DEVELOPMENT AND ASSESSMENT OF PRODUCT MADE FROM FLAX SEEDS AND PUMPKIN SEEDS FOR HYPERCHOLESTEROLEMIC PATIENTS.

A Dissertation-II Report Submitted

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By
BHARTI CHARAK

(Registration No.: 11306817)

Under the Supervision of

Ms. Harsha Hirdyani (17816)

Assistant Professor (Nutrition and Dietetics)

School of Agriculture

LOVELY PROFESSIONAL UNIVERSITY

PHAGWARA, PUNJAB, INDIA

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LOVELY PROFESSIONAL UNIVERSITY

PUNJAB, INDIA

CERTIFICATE

This is to certify that **Bharti Charak** has personally completed M.Sc. dissertation entitled **"Development and assessment of product made from flaxseeds and pumpkin seeds for hypercholesterolemic patients"** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of dissertation has ever been submitted for any other purpose at any university.

The project report is appropriate for the submission and the partial fulfillment of the conditions for the evaluation leading to the award of Master of Nutrition and Dietetics.

DATE:

Ms. Harsha Hirdyani (17816)

Assistant Professor (Nutrition and Dietetics)

School of Agriculture

Lovely Professional University, Phagwara

Signature of Supervisor

ABSTRACT

Hypercholesterolemia is one of the most widespread cardio vascular problem in the world affecting more than millions of people. Also known as high or raised cholesterol levels, hypercholesterolemia thus increases the risk of heart attacks, strokes and plaque formation.. It is therefore a serious concern for population.

Objectives: Development of quality products for hypercholestrolemic patients using flax seeds and pumpkin seeds and Organoleptic and nutritional evaluation of the developed products.

Methods: Flax seeds and Pumpkin seeds were washed and dried by oven drying and using these seeds in different concentrations, food samples products (Chikki) were formulated and Organoleptic properties of the recipes were assessed using 9- point Hedonic scale by a panel of 10 judges. Biochemical estimations were done to detect its, moisture, ash, crude fiber , crude fat, protein, peroxide value, carbohydrate and total energy using AOAC methods (2010) and various other methods.

Results: Organoleptic evaluation resulted in the acceptance of almost all the five samples but highly accepted was sample EF (pumpkin seeds and peanuts). Incorporation of Flax seeds and Pumpkin seeds gave value added results such : Moisture content was highest in EF and GH(4.2% , 3.8%). Ash content was highest in EF and GH (1%, 1.5%). Crude fat was highest in GH-23%. Crude Fiber was highest in the DE (2.6%). Protein content was highest in EF-(14.2%) .Carbohydrate was highest in BD-67.3% and Total energy content was highest in 496.5 kcal-AC. The least retention was seen in the following samples: Moisture- 3% in AC ,Ash- 1% in EF, DE and AC, Crude fat- 16% in EF, Crude fiber- 1% in BD ,Protein -10.1% in BD, Carbohydrate-58.6% in EF and Total energy-467.1 Kcal in BD.

Conclusions: Considering the health benefits and nutrient composition of Flax seeds and Pumpkin seeds, they can therefore significantly contribute to reduce the morbidity of Hypercholesterolemia and can be used as a natural fortifier and can be incorporated in different products.

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Above all I offer countless thanks to the almighty as this modest piece of work has been made possible by his immense grace.

Finally, my apologies and thanks to all those who helped me in one or the other way, but remain unnamed.

Bharti Charak

DECLARATION

I hereby declare that the work presented in Dissertation-II entitled "**"Development and assessment of product made from flaxseeds and pumpkin seeds for hypercholesterolemic patients"** is my own and original. The work has been carried out by me at School of Agriculture, Lovely Professional University, Phagwara, Punjab, India under the guidance of **Ms. Harsha Hirdyani (17816),** Asst. Prof. of School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree Master of Science in Nutrition And Dietetics.

Date: **Bharti Charak**

Place: Phagwara, Punjab (India) **Registration No. :** 11306817

I certified that the above statement made by the student is correct to the best of my knowledge and belief.

Date: **Ms. Harsha Hirdyani (17816)**

Place: Phagwara, Punjab (India) Assistant Professor (Nutrition and Dietetics)

School of Agriculture

Lovely Professional University, Punjab.

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INTRODUCTION

"High cholesterol- a concealed weapon. It's deadly. "

-Jarod Kintz

Hypercholesterolemia, defined as excessively high plasma levels of cholesterol, acts as a major risk factor for cardiovascular disease (CVD). Total cholesterol levels are considered to be a major independent risk factor for development of peripheral diseases, therefore major focus is towards the evaluation of the impact and mechanisms of cholesterol lowering therapies and interventions for cardiovascular outcomes. Too much cholesterol, however increases a person's risk of developing heart disease.

Cholesterol is a fatty substance that occurs in oneself. It helps in several functions. Cholesterol is required to make the walls surrounding the body's cells and is the basic material that is converted to certain hormones. Body makes all the cholesterol that is needed. Only a small amount of fat in the diet is required to make enough cholesterol to stay healthy. The fat and cholesterol being eaten are absorbed in the intestine and transported to the liver. The liver also plays an important role in converting fat , and thus transports the cholesterol into bloodstream which can be seen in the blood. (Stapleton *et al.,* 2010)

Cholesterol travels through the blood attached to protein and this package is called lipoprotein. Lipoproteins are classified by their density: very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) (Biggerstaff KD, Wooten JS 2004). All the lipoproteins carry cholesterol, but the elevated levels of the lipoproteins other than HDL (termed non-HDL cholesterol), particularly LDL-cholesterol is associated with an increased risk of atherosclerosis and coronary heart diseases (Carmena R *et al., 2004*]. Elevated levels of non-HDL cholesterol and LDL in the blood may be a result of diet, obesity, inherited (genetic) diseases (such as LDL receptor mutations in familial hypercholesterolemia), or the existence of other diseases such as diabetes. (Durrington, P 2003)

People with hypercholesterolemia have a high risk of developing heart disease called coronary artery disease. This condition arises when excess cholesterol in the bloodstream is deposited in the walls of blood vessels, mainly in the arteries which supply blood to the heart (coronary arteries). The improper buildup of cholesterol forms clumps (plaque) that narrow and harden artery walls. As the clumps get bigger in size, they can clog the arteries and restrict the flow of blood to the arteries.

Hypercholestrolemia is the presence of high levels of cholesterol in the blood. It is a form of .hyperlipidemia (elevated levels of lipids in the blood) and "hyperlipoproteinemia" (elevated levels of lipoproteins. This medical condition becomes very severe when HDL (good) cholesterol is found less .If not treated properly, this may eventually lead to a heart attack due to coronary artery disease or a stroke due to narrowed arteries supplying the brain (Durrington, P 2003).

1.1 Prevalance of Hypercholesterolemia:

Nawi Ng *et al* documented the trends in cholesterol and triglyceride levels in different population groups and to estimate the risk of developing hypercholesterolemia and hypertriglyceridemia in Sweden, Vasterbotten County, during 1990–2010. Since 1990, 133,082 people living in northern Sweden, Västerbotten County, who were invited on their 30th, 40th, 50th and 60th birthdays, showed participation in the Intervention Program of Vasterbotten. 34,868 individuals were surveyed for a second time after ten years. As a result the level and prevalence of hypercholesterolemia came down significantly from start till 2007, but there was found an increase during 2008–2010 in men, women. This particular study revealed that people with little education and who live in rural inlands had consistently higher triglyceride level than those who live in the city and have higher educational attainment . At 10-year follow-up; the risk was much higher among the older people, particularly the women. From the year 1990 till 2010, no. of individuals who reported treatment with lipid-lowering agents increased from 1.1% to 9.6% among men and 0.5% to 5.3% among women. About 60% of those treated achieved treatment goals for cholesterol or triglycerides**.**

Ozra Tabatabaei-Malazy *et al*.,2014 focused on assessment of prevalence of dyslipidemia as a major risk facot for CVD . Data was collected on the basis of cut-off points of dyslipidemia used in each study. The estimated prevalence and 95% confidence intervals in 29 eligible articles and one un-published data for hypercholesterolemia, levels of low density lipoprotein cholesterol as high ([LDL-C] [≥ 130 mg/dl]) and low levels of high density lipoprotein cholesterol ([HDL-C] <40 mg/dl in males,and in females <50 mg/dl), in people of iran were 41.6% (36.1-47.0), 46.0% (43.3-48.7), 35.5% (24.0-47.1) and in both the sexes 43.9% (33.4-54.4)was in both rural and urban areas. Hypercholesterolemia, high LDL and low HDL levels of cholesterol were more seen in women, on the other hand hypertriglyceridemia was more seen in men. Abnormalities associated with lipid components were more prevalent in urban residents.

A study was conducted on Indian subjects suffering from hypercholesterolemia. Around 40% youngsters were found to be suffering from the risk factors of hypercholesterolemia. This review aimed at defining the age group for stroke condition in youth, and laid emphasis on condition of stroke in youth on the basis of different population-based studies and details the risk factors and diagnostic approach of different stroke subtypes in young people (Prasad K *et al*., 2010).

1.2 Etiology:

Hypercholesterolemia is typically due to a combination of two factors.

1.2.1 Environmental- Environmental factors include obesity and choices in terms of diet. It includes basically the lifestyle choices of an individual and also caused due to reduced physical activity and also due to wrong eating habits. Also reduced physical activity and more intake of food leads to an increase in weight and therefore more chances of this disease may occur.

1.2.2 Genetic- Its contributions are usually because of the additive effects of genes which are, but also may be due to one or single gene defect such as in the case of familial hypercholesterolemia .A number of secondary causes exist including: diabetes mellitus type 2, obesity, alcohol consumption , nephrotic syndrome, obstructive jaundice, hypothyroidism, ,anorexia nervosa, medications (thiazide diuretics, ciclosporin, glucocorticoids, beta blockers, retinoic acid**)** (Bhatnagar D, Soran H, Durrington PN 2008).

High blood cholesterol levels typically result from a combination of genetic and environmental factors. Various choices including eating pattern, physical activity, and tobacco smoking increases the amount of cholesterol in blood. Additional factors can also affect cholesterol levels. More a person is obese more will be prone to hypercholesterolemia. A small percentage of people with high cholesterol have an inherited form of hypercholesterolemia.

1.3 Manifestations of hypercholestrolemia:

Hypercholesterolemia itself does not show much symptoms, sudden increase of serum cholesterol but can lead to atherosclerosis (Bhatnagar D *et al.,* 2008). Chronically elevated serum cholesterol contributes to formation of atheromatous plaques in the arteries. This can cause narrowing or even complete blockage of the arteries which are involved. Various smaller plaques may also cause rupture and cause a clot to form and obstruct blood flow (Finn AV *et al.,* 2010) .A sudden blockage of a coronary artery results in a myocardial infarction or heart attack. Blockage of an artery supplying the brain can cause a stroke. At this point that tissue ischemia (restriction in blood supply mainly lack of oxygen supply) may manifest as specific symptoms. Insufficient blood supply to the heart may cause chest pain, and ischemia of the eye may manifest as visual impairment. Lack of blood supply to the legs can manifest as calf pain when walking, while in the intestines it may present as abdominal pain after eating a meal (Grundy, S *et al* .,1998),(Durrington, P 2003).

1.4 Treatment of Hypercholesterolemia:

Hypercholesterolemia can be treated by various methods and therefore the treatment depends on the medical condition of the patient.

Diet- It causes a major effect on blood cholesterol but the size of this effect differs substantially between individuals (Howell WH *et al.,* 1997). Intestine involves approximately 50% of non-esterified cholesterol absorption, but depends on the efficiency of uptake and the effect of other dietary components such as plant sterols and fiber content affect absorption (Lichtenstein AH 1990). Moreover, when the dietary cholesterol intake goes down, production (principally by the liver) typically increases. Reductions in fat intake, particularly saturated fats, results in reducing

the blood cholestrol. (Sacks FM, Katan M 2002) Dietary sucrose and fructose can raise LDL cholesterol levels (Schaefer EJ *et al.*, 2009). There are also evidence that inclusion of 2 g per day of plant stanol or sterol esters and 10 to 20 g per day of soluble fiber decrease dietary cholesterol absorption.

Dietary changes can typically achieve reductions of 10 to 15% in blood cholesterol. In strictly controlled surroundings, dietary changes can reduce cholesterol levels by 15 percent. In practice, dietary advice can provide a modest decrease in cholesterol levels and may be sufficient in the treatment of mildly elevated cholesterol. (Tang JL *et al.*, 1998)

Therefore, it was hypothesized that soluble fiber intake can reduce the plant sterols absorption among subjects receiving highly effective therapies for lowering lipid levels. Testing of Fiber intake was carried in two different strategies including, which used high-dose statins or combination of a statin plus a inhibitor of cholesterol absorption. These dosages and drugs were chosen to get similar changes in lipid profile through distinct mechanisms.

Lifestyle changes-

Lifestyle changes including physical activity, alcohol consumption, smoking has known to be beneficial for patients suffering from cardiovascular diseases. Cigarettes and other forms of tobacco may help to increase LDL levels (bad cholesterol), and lower down HDL levels (good cholesterol). In fact, smoking highly increases the risk of heart attacks and strokes.

Exercise helps in reducing the obesity which can ultimately lead to decrease the risk factors of hypercholesterolemia. Recommended for those with high cholesterol include: smoking stoppage, limiting alcohol consumption, increasing physical activity, and maintaining a healthy weight. A diet that emphasizes low-cholesterol foods, restricts saturated fats (Bhatnagar D, Soran H, Durrington PN 2008) **,** and avoids trans fat is also recommended. Although changes in lifestyle, including a prudent diet have been widely recommended for primary or secondary prevention of CVD, intake of soluble fiber-enriched diet, not only for the achievement of lipid goals, but particularly to the balance between phytosterolemia and synthesis of cholesterol. Calorie dense food must be reduced along with a reduction in smoking, consumption of alcohol and also physical activity can be increased to reduce the risks of hypercholesterolemia.

1.4.1 Medications used for treatment:-

Statins (or HMG-CoA reductase inhibitors) – If diet is not effective these are commonly used to treat hypercholesterolemia . However various drugs are only recommended if statins cannot be tolerated (NIHCE, 2008). Statins can reduce total cholesterol by approximately 50% in the majority of people. Also statins play role in decreasing mortality in those who have had previous CVD, they are effective in those with high cholesterol but no other health problems. (Ray *et al*.,2010)**.**

Based on clinical trials, high doses of statins have been recommended to achieve lower levels of LDL-cholesterol. Nevertheless, the use of high doses of statins is not always well tolerated or effective.

1.5 Dietary suggestions for a hypercholesterolemia patient:-

Diet is clearly a cause of hypercholesterolemia but is not the only one. Obesity being considered as the main cause and should be controlled. Fruit and vegetables contains valuable nutrients and also possess a number of plant chemicals (phytochemicals) which provide some protection again problems of heart and cancer.

The point is, to maintain a healthy body and emphasis is laid on attaining good metabolism, therefore a wide range of nutrients are required, for effective functioning. So for best cholesterol results, it's essential to develop healthy eating habits across the board**.** Obesity is considered as a main cause for coronary heart diseases therefore reduction in weight may help in treating the condition. (Tripathy B.B. *et al.*, 2002)

Fat substitutes have been developed to impart the functional and sensory qualities of fat and decrease the quantity of fat in foods to assist in decreasing fat intake. Within the context of a healthy diet that meets contemporary dietary recommendations, fat substitutes, used properly may provide flexibility in treating this condition.

Omega-3 Essential Fatty Acids- these types of fat are essential because our body does not provide them itself. The most important essential fatty acid is known as omega-3 (alpha-linolenic

acid). It found in some vegetable oils like flaxseed oil and fish oils from oily fish like mackerel, salmon, herring, sardines and tuna. In addition, it should be kept in mind the foods which contains "hydrogenated" or "partially hydrogenated" or "trans-fats". For example, there should be limit in consumption of baked goods, and other processed foods which are made with these kinds of fat. Cholesterol levels are increased by a high intake of saturated fat, hydrogenated or Trans fat and other lipid levels, and may cause heart problems. (He Ka *et al* .,2007).

1.5.1 High Fiber, Low-GI Carbohydrates

Carbohydrates are considered as very nutritious and also an excellent source of both soluble and insoluble fiber. The lipid effects of whole grains depend on their soluble fiber content. Barley and oats, but not whole wheat, rye or rice, contain β-glean, a subtype of soluble fiber that becomes gel-like in the intestine and binds bile acids, thereby promoting their fecal loss.

Usually as breakfast cereal, oat diets have been the most studied against control diets in controlled clinical trials and results have mean reductions of total cholesterol have been found reduced to 7.7 mg/dl and of LDL-cholesterol of 7 mg/dl. Increased intake of whole grains of any kind, even those not containing soluble fiber, has been associated to reduce the risk of CHD and other cardio metabolic outcomes (Harris KA *et al* .,2001)

Lipid levels can be lowered with many changes such as lifestyle changes, medications, or a combination of these approaches. A healthcare provider also plays a very vital role and thus gives recommendation of trial of lifestyle changes before suggesting a medication. A change must be made in day to day life and also efforts must be made to reduce total and saturated fat in the diet, losing weight, also increasing physical activities, and eating a diet rich in fruits and vegetables. Flaxseeds in human health has already been done for treating many medical conditions such as constipation. Fiber content of Flaxseeds have also been playing role on blood glucose metabolism and hyperlipidemia in the areas where soy products are not much consumed Flaxseeds must be included as a good source of phyto-estrogens and among various populations with low vegetable oil consumption (Tarpila A *et al*., 2005).

Pumpkin seeds are consumed raw in some parts of the world and some use roasted or cooked, but only at the domestic scale. Richness in nutrient content of Pumpkin seeds such as protein,

fibres, minerals, polyunsaturated fatty acids, they are considered of great value for the food industry. Also, other effects on blood glucose level, immunity, cholesterol, liver, prostate gland, bladder, depression, and parasite inhibition, impaired cognitive functioning have been considered. Thus pumpkin seeds have major role in relieving and reducing human conditions and also have various nutritional properties (Patel S, 2013).

Therefore finding hypercholestrolemia as a major health affecting issue worldwide, a dietary treatment including the incorporation of flax seeds as well as the pumpkin seeds will be used in this study. Therefore the main objective of this study is to develop the fibre rich products which are rich in omega-3 fatty acids mainly for hypercholesterolemic patients and to obtain the products by incorporating the flax seeds and pumpkin seeds.

CHAPTER- 2

TERMINOLOGY

CVD- Cardiovascular diseases

VLDL- Very low density lipoprotein

LDL- Low density lipoprotein

HDL- High density lipoprotein

HDL-C- High density lipoprotein cholesterol

LDL-C- Low density lipoprotein cholesterol

CHD- Coronary heart disease

PVD-Peripheral vascular disease

CAD- Coronary artery disease

AHA- American heart association

TC- Total cholesterol

ALA- Alpha linolenic acid

LDLr- Low density lipoprotein receptor

NZW- New Zealand white rabbit

PSO- Pumpkin seed oil

UNSAP- Unsaponifiable

TGs- Triglycerides

PF- Pumpkin flour

º - Degree

C- celcius

BSA- Bovine serum albumin

%- Percentage

Gm- grams

Kg- Kilogram

Meq- Milli equivalent

Mg/dl- Milli gram per deci litre

CHAPTER- 3

REVIEW OF LITERATURE

A systematic review is a literature review which focuses on a research question, trying to identify, appraise, select and synthesize all high quality research evidence relevant to that question.

Hypercholesterolemia can be defined as very high plasma cholesterol levels, and there is a strong risk factor for cardiovascular diseases. Total cholesterol levels above 200 mg/dl have been related as an independent risk factor for development of peripheral vascular (PVD) and coronary artery disease (CAD). Hypercholesterolemia is considered to be major cause for stroke and atherosclerosis. However, some hypercholesterolemia treatment methods that have been widely used, including pharmaceutical therapies which helps in decreasing circulating cholesterol by preventing either its formation in the liver or its absorption. The careful clinical characterization of patients with genetic forms of severe hypercholesterolemia has played a vital role in the historic linkage of hypercholesterolemia to atherosclerosis. The drugs and the various other treatments given for such cases have resulted out in reduction of the plasma cholesterol levels and also reduce total mortality**.** (Phoebe A Stapleton *et al.,* 2010)

4.1 Prevalence of Hypercholesterolemia:-

Prevelance worldwide :

Hypercholesterolemia is considered as a world wide problem. Three major modifiable coronary risk factors have been seen today smoking, hypertension, and hypercholesterolaemia, the last is the one most prevalant to the populations as a whole.

Manolio TA *et al* in 1992 carried out a hospital based study in Taiwan which documented the prevalence of Hypercholesterolemia in Middle-aged Taiwanese adults. The aim of this study was to explore the prevalence of hypercholesterolemia and its related factors in middle-aged Taiwanese adults. This was a cross-sectional hospital-based study. Elevated total serum cholesterol was seen as one of the modifiable risk factors of cardiovascular disease (Soons KR, *et al*). A reduction in total serum cholesterol by 1% had been shown to correlate with a 2%reduction in the risk of cardiovascular disease **.** The prevalence of hypercholesterolemia (≥

6.22 mmol/L) was11.5% in men and 10.0% in women , a condition which calls for either dietary or pharmacological intervention.

Lloyd-Jones D, *et al* in 2009 considering hypercholesterolemia as a strong risk factor for cardiovascular disease (CVD) ,conducted a survey and documented that the mean total serum cholesterol for Americans over the age of 20 was 199 mg/dl, approximating the American Heart Association (AHA) recommended level of 200 mg/dl**.** Unfortunately, 16% of adults were found to have total cholesterol levels of more than 240 mg/dl, a level considered by the AHA to carry twice the CVD risk of those individuals at the desired level

Phoebe A Stapleton *et al* in 2010 considered high total cholesterol levels to be a major independent risk factor for development of peripheral vascular disease and coronary artery disease, considerable attention had been directed toward evaluating the impact and mechanisms of cholesterol lowering therapies and interventions for cardiovascular outcomes . Cholesterol had been shown to interrupt and alter vascular structure and function as it builds within the lining of the vascular wall, and could affect the endothelial lining which further leaded to various problems like plaque etc. With specific relevance to the microcirculation, it was clear that cellular dys functioning leaded to various problems. On investigation it was clear that due to some of the therapies given the mortality was reduced to some extent.

Prevalence studies were conducted in portugese on hypercholesterolemia and included 53,445 individuals overall, with sample size lower than 1000 in most of the individual studies. The major objective of this study was to make their country men aware of hypercholesterolemia and to find out that how much work was done to treat it. The most frequently used criterion for hypercholestrolemia was > 200 mg/dL, with the mean level being higher in most studies. They calculated the mean prevalence for several cut-off points (190 mg/dL: 63.8%; 200 mg/dL: 56.7%; 240 mg/dL: 31.7%; 250 mg/dL: 21%). Due to the heterogeneity of the data, these results were interpreted with caution, even though they were consistent across individual studies. The prevalence of hyper cholestrolemia was majorly noticed here. (Costa J *et al.,* 2003)

Prevalance in India

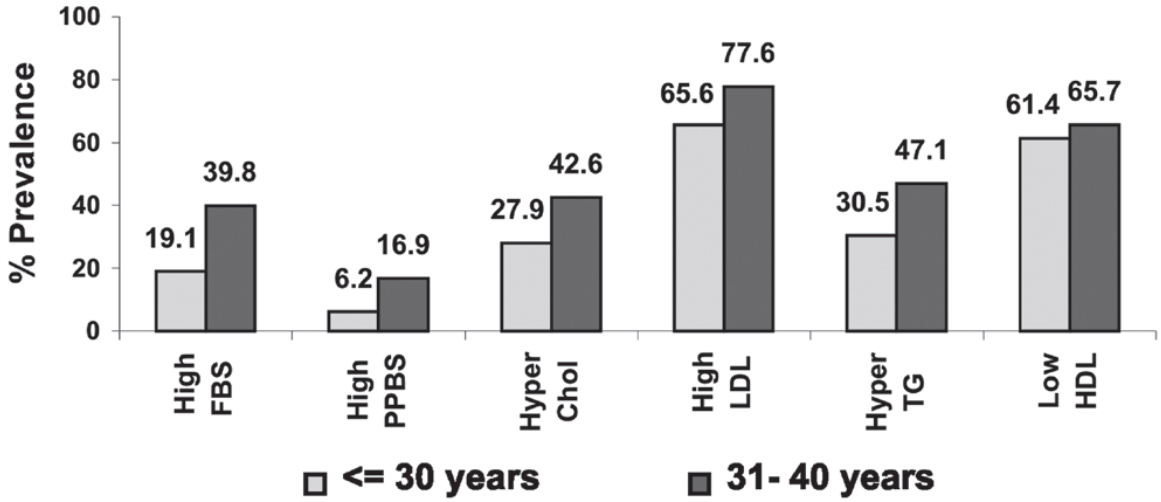
Dewan BD in 1974 published the first Indian study using the Minnesota code in 1974, which reported CHD prevalence of 2.28 % among males and 1.73% among females in rural Maharashtra. Later during 1987-88 Chadha *et al* carried out a study in the rural areas of Haryana and reported a lower prevalence of symptomatic CHD among men (0.74%) and women (0.51%) while total prevalence was higher (2.71%) when silent CHD cases were also included. The urban sample had a higher symptomatic CHD prevalence (3.19%) compared to rural areas (0.59%). (World Health Report 2002).

Bandana S in 2002 investigated the dietary pattern and lifestyle having a major influence on the cardiovascular and metabolic diseases as a major factor. The prevalence of borderline high cholesterol level (200- 239) and high cholesterol (>240) was 21.1 % and 9.4 % among select tribal populations. It was found that 71.7% were non-vegetarian and 28.3% were vegetarian out which 82.6% were males and 63.2% were females. It was observed that males (10.7%) had high cholesterol level as compared to females (8.4%). Females were slightly more at borderline high cholesterol level as compared to males. Working group (9.8%) had high level of cholesterol level as compared to non-working group (6.7%). Overall consumption of mustard oil was 100% among these populations. That was mainly the reason for having less prevalence rate of high cholesterol level as compared to rural and urban populations.

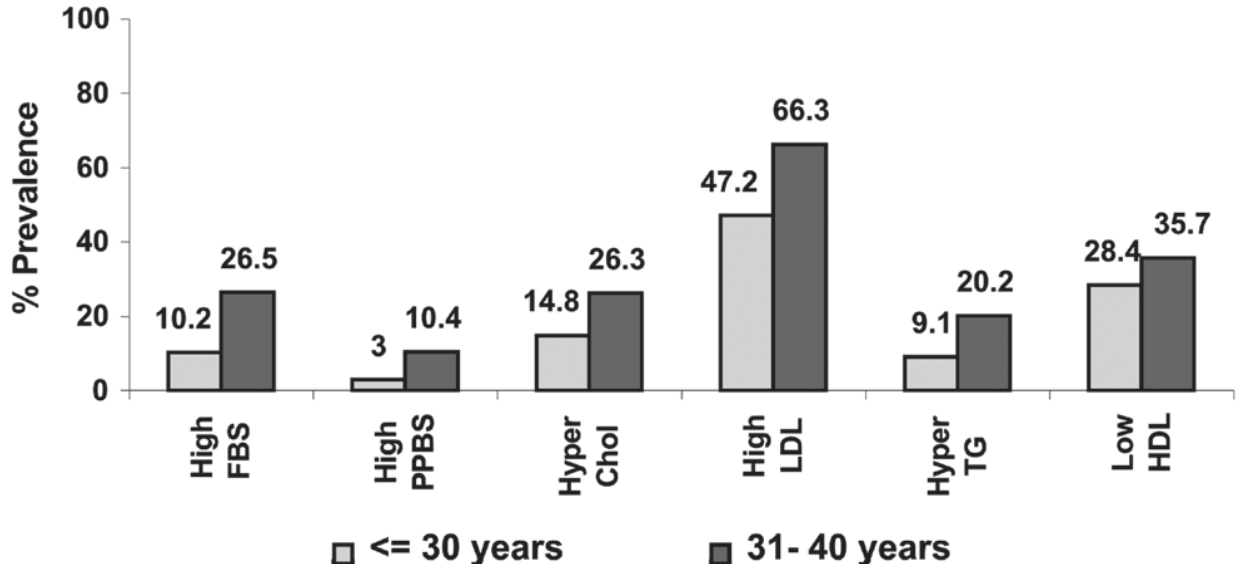
Another study by AM Sawant *et al* in 2006 documented that prevalence of dyslipidemia in young adult indian population and the cardiovascular diseases (CVD) were the major cause of morbidity and mortality in the society with dyslipidemia contributing significantly to atherosclerosis. The study was conducted for a period of one year – from 1st January 2006 to31st December 2006. Around 1805 subjects with ≥ 40 age group were selected from a population of approximately 9000 urban dwellers who had attended annual general health check ups in P. D. Hinduja National Hospital and Medical research Center. Health status was evaluated by physical check ups, complete fasting lipid profiles and blood glucose levels.

Among participants who had a total Cholesterol (TC) concentration ≥ 200mg/dl, 38.7% were males and 23.3% were females. High density lipoprotein cholesterol (HDL-C) was abnormally

low in 64.2% males and 33.8% in females. The increase of prevalence of hypercholesterolemia and hypertriglyceridemia was more prominent in 31-40 age group than in ≤ 30 age group. The low percentage of adults with controlled lipid concentrations suggested that there was a need for awareness programs for the prevention and control of Dyslipidemia and impaired blood sugar levels. (Fig 4.1, 4.2)



Graph :4.1- Age specific prevalence of dyslipidemia & impaired blood glucose among males of age ≤30 years and 31 to 40 years



Graph :4.2 Age specific prevalence of dyslipidemia & impaired blood glucose among females of age ≤30 years and 31 to 40 years.

4.2 Dietary approach for the treatment and prevention :-

A major part of the circulating cholesterol originates from diet, and restricting cholesterol intake may reduce blood cholesterol levels, there are various other links between the dietary pattern and cholesterol levels. Lowering down the cholesterol content of body various factors like lifestyle changes, doing physical exercise, eating healthy food can help reducing cholesterol of your body. Various studies documented below have shown the dietary effects on reduction of hypercholesterolemia. In addition to eating a healthy diet , low in saturated fat, with plenty of whole grains, fruits, and vegetables , some specific foods and supplements may help lower cholesterol. (Ellegard LH *et al* .,2007)

Fiber - LDL cholesterol and increased lipid levels can be reduced by soluble fiber (which is found in oat bran, wheat, barley, apples, psyllium, and flaxseed) . Weight can be reduced by including fiber in the diets because they provide a feeling of fullness. Doctor or health professionals always suggests to include fiber in the diets. Also fiber can be considered as a supplement. 30 - 38 g of fiber per day must be taken by males and 21 - 25 g per day by females.

Beta-glucan – It is a kind of soluble fiber which is present in oat bran and various other plants. It also reduces LDL cholesterol slightly, because of which oat bran is considered as a cholesterol-lowering food.

Alpha-linolenic acid (ALA) - ALA is another omega-3 fatty acid that plays a vital role in protecting the heart.

Beta-sitosterol – It is another plant sterol, which is a compound that can inhibit cholesterol absorbed by the intestines. Various scientific studies have depicted that beta-sitosterol plays a role in lowering "bad" LDL cholesterol levels in the body. Beta-sitosterol may decrease the contentof vitamin E and beta-carotene which is absorbed by the body, so extra intake of vitamin E and beta- carotene must be considered.

Polyphenols –These are the chemical substances which are found in plants that possess antioxidant properties. Test tube, animal, and some population-based studies suggest that the

flavonoids and catechins (found in high concentration in grape juice, and red wine) may help in reducing the risk of atherosclerosis by protecting from the destruction caused by LDL cholesterol. However, to confirm these findings various human based studies would be done.

The potential role of soluble fibre in the treatment of hypercholesterolaemia:

Dietary fibers play an important role in the normal functioning of the gut, as well as in maintaining the cholesterol levels in humans. Soluble fibers appear to have their effect by reducing the rate of absorption from the small intestine. Furthermore benefits of soluble fibre also appear in relation to the incidence of certain cancers. Soluble fibers therefore have good reasons to have a range of metabolic health benefits. Their potential uses, mechanisms of action and means of incorporation into diets require further exploration. (Mishra A *et al*., 2010)

Harris KA *et al* in 2001 conducted another study to see the lipid effects of whole grains depending on their soluble fiber content. Barley and oats, but not whole wheat, rye or rice, contain β-glucan, a subtype of soluble fiber that becomes gel-like in the intestine and binds bile acids, thereby promoting their fecal loss. Oat diets, usually as breakfast cereal, have been the most studied against control diets in controlled clinical trials and results have demonstrated mean reductions of total cholesterol of 7.7 mg/dl and of LDL-cholesterol of 7 mg/dl. Interestingly, increased consumption of whole grains of any kind, even those not containing soluble fiber, had been consistently associated with a reduced risk of CHD and other cardio metabolic outcomes, probably because all whole grains are seeds rich in beneficial nutrients and phytochemicals which have a very major role in our body.

Ullrich IH in 1987 focussed on epidemiologic studies of cardiovascular mortality rates in different countries that had suggested that dietary fiber may play a protective role. Within a similar population, a large intake of fiber was associated with a lower relative risk of death from coronary heart disease. Dietary fiber may be separated into at least two types: insoluble, which includes cellulose, hemicellulose, and lignin; and soluble, including pectin and gums. While some studies have shown continued improvement over a period of months, this has not been uniformly found. Both normal and elevated triglyceride levels appear to be more resistant to change with dietary fiber. An increase of dietary carbohydrate as a source of fiber may be

associated with an increase in triglyceride levels. Fiber may, however, offer some protection against an increase in cholesterol and triglyceride levels in subjects fed diets containing large amounts of sucrose. Although rats fed oat bran, guar gum, or pectin had lower levels of hepatic and blood triglycerides, humans with hypercholesterolemia fed oat bran or guar showed no effect on their triglycerides.

4.3 Significance of flax seeds:-

Flaxseed is a smooth, flat and reddish-brown in color is native to the region extending from the eastern Mediterranean to INDIA also called as(linseed). Whole flaxseeds contain 28% dietary fiber (7-10%soluble fiber, 11-18% insoluble fiber); 40% fats (57% of omega 3 fatty acids) and 21% proteins. it is also the richest source of phtoestrogens- lignans42.Consumption of flaxseeds have shown to reduce total and LDL cholesterol as well as platelet aggregation. The major bioactive components responsible for hypolipidemidic action of flax seeds are dietary fiber. (Pan A *et al* and Patade A *et al* 2013)

Table- 4.1.Composition of Flaxseed

Component analysis	Concentration
Moisture (%WW)	7.7
Ash (%DW)	3.4
Crude fat (%)	41.00
Crude protein (%DW)	20
Dietary fibre (%DW)	28
Available carbohydrate (%DW)	29
Energy value (kcal per 100 g)	450

(Morris D.H, 2003).

Table- 4.2 Fatty acids composition of Flax seeds

Fatty acids	Concentration%
Palmitic acid (C16:0)	6.58
Stearic acid (C18:0)	4.43
Oleic acid (C18:1)	18.51
Linoleic acid (C18:2)	17.25
Linolenic acid (C18:3)	53.21

(Popa V.M *et al*, 2012).

Fig:4.1: Flaxseeds

The increased use of omega (ω)-3 fatty acids was a powerful example of nutritional strategy that may produce significant cardiovascular benefits. Marine food products had provided the traditional dietary sources of ω-3 fatty acids. It is one of the richest sources of the plant-based ω-3 fatty acid, alpha-linolenic acid (ALA). Based on the results of clinical trials, epidemiological investigations and experimental studies, ingestion of ALA has been suggested to have a positive impact on CVD. Because of its high ALA content, the use of flaxseed has been advocated to combat CVD. The purpose of the present review was to identify the known cardiovascular effects of flaxseed and ALA and, just as importantly, what is presently unknown. (Delfin Rodriguez-Leyva, MD, PhD *et al* in 2010)

Kristensen *et al* in 2012 examined the effect of flaxseed dietary fibers in different food matrices on blood lipids and fecal excretion of fat and energy in a double-blind randomized crossover study with 17 subjects. Three different 7-d diets were tested: a low-fiber control diet (Control), a diet with flaxseed fiber drink (3/day) (Flax drink), and a diet with flaxseed fiber bread (3/day) (Flax bread). Total fat and energy excretion was measured in feces, blood samples were collected before and after each period, and appetite sensation registered 3 times daily before main meals. Compared to control, Flax drink lowered fasting total-cholesterol and LDL-cholesterol by 12 and 15%, respectively, ($p < 0.01$), whereas Flax bread only produced a reduction of 7 and 9%, respectively ($p < 0.05$).. Both Flax drink and Flax bread resulted in decreased plasma total and LDL-cholesterol and increased fat excretion, but the food matrix and/or processing may be of importance.

Simbalista *et al*., in 2010, used partially defatted flaxseed meal at a dose equivalent to 25 g/day of whole seed. The smallest study included 10 subjects, while there were 179 subjects in the largest study. The direction of effect was highly consistent towards a reduction in total cholesterol (100%) and LDL cholesterol (100%) levels when flaxseed was consumed. The main endpoints considered were total cholesterol and low-density lipoprotein (LDL) cholesterol. These were recognized as risk factors or biomarkers for heart disease. The direction of effect was highly consistent towards a reduction in total cholesterol (100%) and LDL cholesterol (100%) levels when flaxseed was consumed. However, a very low proportion of studies showed a statistically significant reduction in total cholesterol (25%) and LDL cholesterol (0%) levels. (Dodin *et al*., 2005)

Chantal M.C. Dupasquier and Grant N. Pierce examined the effects of flaxseed supplementation on these parameters animal models, the hypercholesterolemic rabbit and more recently, the cholesterol fed, low density lipoprotein receptor (LDLr-/-) deficient mouse in 2007**.** Male New Zealand White (NZW) rabbits were fed a diet containing flaxseed in the absence or presence of dietary cholesterol for a period of 6 - 16 weeks. The omega-3 fatty acid content of the flaxseed may provide an anti-inflammatory action that could inhibit atherogenesis.. Dietary supplementation with ALA from flaxseed oil has been demonstrated to reduce circulating levels of several atherogenic and inflammatory markers . This mechanism played an important contributory role in the anti-atherogenic effects of flaxseed in this model and, more importantly, it had this action in humans as well.

4.4 Significance of pumpkin seeds:-

Table 4.3: Proximate composition of Curcubita

Component analysis	Concentration
Moisture (%WW)	5.00
Ash (%DW)	5.50
Crude protein (%DW)	27.48
Crude fibre (%DW)	1.00
Available carbohydrate (%DW)	28.03
Energy value (kcal per 100 g)	564

(Elinge C.M *et al* 2012)

Table 4.4 Fatty acids composition of Pumpkin seeds

Fatty acids	Concentration%
Palmitic (C16:0	10.68 ± 0.42
Palmitoleic (C16:1)	0.58 ± 0.14
Stearic (C18:0)	8.67 ± 0.27
Oleic (C18:1)	38.42 ± 0.37
Linoleic (C18:2)	39.84 ± 0.08
Linolenic (C18:3)	0.68 ± 0.14

(A. Gohari Ardabili *et al,* 2011)

Fig 4.2: Pumpkin seeds

Pumpkin belonging to the family Cucurbitaceae, is a perennial plant and is consumed traditionally in a variety of foods such as fresh or cooked vegetables, as well as being stored frozen or canned. Undoubtedly, pumpkin seeds are quite beneficial but still the untapped potential of these seeds is yet to be explored.

Pumpkins are rich in water, vitamins, antioxidants and carotene (provitamin A) that protects the body and prevents the premature aging. Pumpkins are also poor in total solids and in calories, which means that they are adequate for low calories regimes, and they are often recommended in diets. This vegetable is also known for the properties of its seeds, that are rich in fat, protein, thiamin, niacin and various minerals and when crushed provide a pulp with medicinal power

which acts as anti-inf ammatory, diuretic and emulsifier that helps in fever treatment, ear pain, inflammation of the urinary and prostate. (F. Henriques *et al.,* 2012)

Elinge C.M *et al* in 2012 conducted a study in which Pumpkin seeds were analysed for their nutritional and anti-nutritional composition, the results obtained were; moisture content (5.00%), ash (5.50%), crude lipid (38.00%, crude fibre (1.00%), crude protein (27.48%), Available carbohydrate (28.03%) and calorific value (564kcal/100g). Elemental analysis shows that potassium is the most abundant element in the sample (273mg/100g) and manganese is least (0.06mg/100g).The anti-nutritional parameters analysed are; phytate (35.06 mg/100g), oxalate (0.02±0.10mg/100g), hydrocyanic acid content (0.22±0.04mg/100g) and nitrate (2.27±002mg/100g). The result shows that the pumpkin seeds if properly utilized can serve as good source of minerals.

Glew RH *et al* in 2006 described pumpkin as a source of the mineral zinc, and the World Health Organization recommended their consumption as a good way of obtaining this nutrient. To maximize the amount of zinc from your pumpkin seeds, it was recommend to consider purchasing them in unshelled form. Although recent studies had shown there to be little zinc in the shell itself (the shell is also called the seed coat or husk), there is a very thin layer directly beneath the shell called the endosperm envelope, and it is often pressed up very tightly against the shell. Zinc is especially concentrated in this endosperm envelope. Because it can be tricky to separate the endosperm envelope from the shell, eating the entire pumpkin seed—shell and all—will ensure that all of the zinc-containing portions of the seed will be consumed. Whole roasted, unshelled pumpkin seeds contain about 10 milligrams of zinc per 3.5 ounces, and shelled roasted pumpkin seeds (which are often referred to pumpkin seed kernels) contain about 7-8 milligrams.

S.Y. Al-Okbi *et al* conducted a study with objective to evaluate the cardiovascular protective effect of Egyptian and European pumpkin seed oil (PSO) in hypercholesterolemic rats. Tocopherols, fatty acids and unsaponifiable matter (UNSAP) were assessed in both oils. The results showed that α-tocopherol was 108 and 273, γ-tocopherol was 3.95 and 0 and δ-tocopherol was 0 and 1.58 mg·100 g-1 oil of the Egyptian and European, respectively. Analysis of fatty acids revealed the presence of linoleic acid as the major fatty acid in both oils. Feeding a

hypercholesterolemic diet produced a significant increase in plasma total cholesterol (T-Ch), triglycerides, low density lipoprotein cholesterol, T-Ch/HDL-Ch, TGs/HDL-Ch and malondialdehyde and a significant reduction in high density lipoprotein cholesterol (HDL-Ch), vitamin E, and adiponectin. Rats fed on hypercholesterolemic diet with either oil showed a significant improvement in all biochemical parameters.

4.4.1 Anti-hypercholesteromia effect:-

Pumpkin seed oil had been found to exhibit anti-hypercholesteromic effect (Al- Zuhairu *et al.*, 1997; Çelik *et al.*, 2011). The presence of unsaturated fatty acids such as oleic acid and linoleic acid in pumpkin seed reduced cholesterol level in rats (Takada *et al.*, 1994). This ultimately resulted in higher absorption of triglyceride rich lipoprotein (very low density lipoprotein, VLDL) and chylomicron in tissues other than liver to promote their breakdown, thereby reducing triglyceride levels. As cholesterol is very essential for lipoprotein biosynthesis and LDL's contain the highest level of cholesterol, LDL is likely to deplete followed by a reduction in cholesterol levels (Sedigheh *et al.*, 2011).

Abuelgassim O *et al* in 2011 conducted another study on the Effect of Pumpkin (*Cucurbita Pepo L*) Seeds and L-Arginine Supplementation on Serum Lipid Concentrations in Atherogenic Rats. The present study aimed to examine the effect of pumpkin (*Cucurbita pepo L.*) seeds supplementation on atherogenic diet-induced atherosclerosis. The findings suggestd that pumpkin seeds supplementation had a protective effect against atherogenic rats and this protective effect was not attributed to the high arginine concentrations in pumpkin seeds.

Amin T, Thakur M in 2013 carried out a study on cucurbita mixta (pumpkin seeds).This study aimed at summarizing the various health benefits of pumpkin seeds along with their nutritional composition. Also aimed to explore the health benefits of pumpkin seeds so that the awareness for nutritive and quality food by health conscious population can be met. This literature supported that more research could be done to investigate the unexplored and under-exploited potential of the plant. This crop contributed to food sector, nutrition, dietary and culinary diversification, health and income generation. The purpose of this study was to discuss various

medicinal and nutritional properties of pumpkin seeds that can further impact many research developments and further can prove as a potential source of functional foods.

Nyam KL,2013 determined the proximate composition, functional properties and antioxidant activity of pumpkin seeds and rind. Besides, the effects of dietary fibre in pumpkin seeds and rinds on bread qualities and properties were evaluated**.** Formulations for bread substituted with 0%, 5% and 10% pumpkin seed and rind, respectively were produced. Sensory evaluation of the prepared bread samples for such attributes as appearance, aroma, flavour, texture and overall acceptability was undertaken. The physical properties of the bread samples, including dough expansion, loaf volume, crumb colour and bread texture, were determined. Proximate analysis and determination of antioxidant activity of the bread samples were also conducted. Crude fibre of the pumpkin seeds and pumpkin rinds was high at 31.48% and 14.83%, respectively. A 5% level of pumpkin rind bread gave the best overall acceptability and sensory attributes, followed by 5% pumpkin seed bread. Total dietary fibre, total phenolic compound and DPPH radical scavenging activity in breads substituted with 5% pumpkin seed and 5% pumpkin rind flour were higher than the values in control bread. Pumpkin seeds and rinds can be used as dietary fibre sources in bakery.

See, E.F in 2007 studied the physico-chemical and sensorycharacteristics of bread supplemented with four different levels (control, 5%, 10%, and 15%) ofpumpkin flour. The physical (weight, loaf volume, specific volume and oven spring) and chemical (moisture, protein, fat, fibre and ash) attributes were determined in the raw pumpkin, pumpkin flour (PF), control and supplemented breads. Sensory attributes were conducted on the control and supplemented breads. Increasing the level of substitution from 5% to 15% pumpkin flour significantly ($p<0.05$) increased the ash and crude fiber. However, there was a significant decrease ($p<0.05$) in protein and fat content. Loaf volume and specific volume of treated bread were significantly different ($p<0.05$) to that of the control treatment. Sensory evaluation results indicated that bread with 5% PF was rated the most acceptable and was not significantly different in terms of acceptability compared to the control.

CHAPTER-4

RATIONALE AND SCOPE OF THE STUDY

Taking hypercholesterolemic patients into major consideration various properties of flax seeds and pumpkin seeds were studied and therefore a product was formulated. Various health benefits are highlighted under-

Flax seeds was introduced recently and is found to have a very major effect in treating hypercholesterolemic conditions. Comparing to the seasame seeds, they posses several times as much as lignans. On comparison of flax seeds with other common eaten foods, especially in terms of polyphenol content, flex seeds rank 9^{th} among 100 of common consumed food. Flax seeds are also a rich source of fibre. Various fruits like blueberry our vegetables like olives which have a high content of polyphenol antioxidants, flax seeds therefore turn out to be having significantly higher polyphenol content. The content of fibre and the benefits of antioxidants present in the flax seeds have been therefore associated with the prevention of many problems and also play a major role in reducing insulin content. (Madhusudhan B 2009)

These are the seeds which posses a wide range and variety of antioxidants such as phytoneutrients, phenolics, phytosterols, etc. and these act as neutraceuticals. Pumpkin seeds are also said to be a good source of various micronutrients and macronutrients such as (phosphorous, magnesium, manganese) etc. also act as a good source of minerals like zinc, iron, etc. They are also rich in protein and antioxidants where thereby play a major role in reducing the cholesterol levels and also treating various other health issues. (Xanthopoulou MN *et al.,* 2009)

Pumpkin seeds also provides valuable amount of carotenoids and ascorbic acids. Foods containing beta-carotene may also reduce certain types of concerns and these pumpkin seeds provides significant protection against heart diseases. The Spanish pumpkin was reported higher in terms of carotenoids content as compared to other varieties of pumpkin. (See E.F *et al* .,2007) Therefore considering all the health benefits of flax seeds and pumpkin seeds, they will be used for the product development for hypercholesterolemic patients.

CHAPTER-5

OBJECTIVES OF THE STUDY

1) Development of quality products for hypercholestrolemic patients using flax seeds and pumpkin seeds.

2) Organoleptic evaluation of the developed products.

3) Nutritional evaluation of the developed products.

4) Shelf life study of the products.

Target group- Hypercholesterolemic patients

CHAPTER- 6

MATERIALS AND RESEARCH METHODOLOGY

EQUIPMENT

1. Spectrophotometer
2. Muffle furnace
3. Dessicator
4. Hot air oven
5. Centrifuging machine

MATERIAL USED

1. Flax seeds
2. Pumpkin seeds
3. Peanuts
4. Jaggery

METHODOLGY:

The present investigation entitled **"Development and assessment of product made from flaxseeds and pumpkin seeds for hypercholesterolemic patients"** was conducted in the Post-graduate laboratory of **Department of Food Technology and Nutrition, Lovely professional university, punjab**.

The most fundamental step in research is to develop a systematic plan for investigation. Hence this chapter contains relevant information pertaining to the research design. It includes the methodological aspect such as selection of tools, procedure of data collection and other techniques of statistical analysis in order to achieve all objectives of the present investigation.
The present study was conducted in the following phases:

1 Procurement of materials

2 Drying of flax seeds and pumpkin seeds

3 Selection of snacks

4 Product development

5 Organoleptic evaluation

6 Biochemical evaluation

7 Statistical analysis

6.1 Procurement of materials

Flax seeds and pumpkin seeds were purchased from the local market of Ludhiana because of its easy proximity, so each of these seeds were purchased respectively.

6.2 Drying

These seeds were seperately arranged in the Oven tray forming one single layer. The oven was pre heated to 40˚C and the tray was placed inside. The temperature was maintained at 60º C and the seeds were dried for 2hrs. These seeds were sufficiently dried till they became crispy and brittle to touch.

6.3 Standardization of the recipes

A standardized recipe should be such that it produces identical results whenever tried under the conditions specified. Accordingly all the variables such as the ingredients, cooking temperature, duration of cooking, the quantity of oil used were controlled. This was designated as the 'basic recipe' and served as 'control' for the purpose of the comparison. The recipe was standardized for the control peanut chikki and then subsequently for flax seeds and pumpkin seeds chikki.

6. 4 Selection of snacks

Snacks generally form an integral part of meals. Healthy snacks are important for both adults and children. Consumption of nutritious snacks help in preventing various health problems, adding more vitamins, minerals and micronutrients to a person's diet. For the present study snack selected for the incorporation of the flax seeds and pumpkin seeds is **"Chikki"** . This sweet snack is selected as it is a finger picked food and can be consumed by people belonging to all the age groups. It is an innovative Indian snack specially for hypercholestrolemic patients.

Pumpkin and flaxseeds chikki had been modified form of traditional Indian sweet snack "peanut chikki". The traditional dish typically consisted of peanut and jaggery in which flax seeds and pumpkin seeds were incorporated as the new product, in order to prepare the desired product which can be suitable for Cardiovascular Diseases.

6.4.1 Method of preparation

Chikki is a sweet snack originated from India. While its preparation the amount of ingredients added should equally be balanced. Its quality depends upon its texture, taste and appearance and also the storage conditions must be optimum.

6.4.2 Method of preparation for Peanut chikki. (BD)

Ingredients	Amounts
Peanuts	50g
Jaggery	50g
Oil	1 tsp

Method-

1. Jaggery was crushed and made into syrup with addition of small amount of oil.
2. The clear jaggery syrup was heated until the temperature reached 145ºC and immediately pre weighed, roasted and dehusked peanuts were added and mixed thoroughly till the nuts get coated with jaggery syrup.
3. Hot mass was then transferred on to a wooden board which was already smeared with oil.
4. The mass was then spread uniformly with help of a roller.
5. Vertical and horizontal lines were marked with a knife or cutter to make uniform pieces and then cooled to room temperature.

6.4.3 Method for preparation of flax seed and pumpkin Chikki

Chikki was developed using the flax seeds and pumpkins seeds and were then incorporated in the basic reciepe at different concentrations. A total of five samples were prepared using jaggery as a standard and seeds were incorporated at different concentrations.

Sample GH: (Flax seeds and peanuts)

Ingredients	Amounts
Flax seeds	25g
Jaggery	50g
Peanuts	25g
Oil	1tsp

Method:

The method used in preparation of flax seeds and peanuts chikki was same as depicted in **6.4.2.**

Sample DE: (Flax seeds only)

Ingredients	Amounts
Flax seeds	50g
Jaggery	50g
Oil	1tsp

Method:

The method used in preparation of flax seeds chikki was same as depicted in **6.4.2.**

Sample EF: (Pumpkin seeds and peanuts)

Ingredients	Amounts
Pumpkin seeds	25g
Jaggery	50g
Peanuts	25g
Oil	1tsp

Method:

The method used in preparation of pumpkin seeds and peanut chikki was same as depicted in **6.4.2.**

Sample AC: (Pumpkin seeds only)

Ingredients	Amounts
Pumpkin seeds	25g

Jaggery	50g
Oil	1tsp

Method:

The method used in preparation of pumpkin seeds chikki was same as depicted in **6.4.2**

Ingridients	**BD**	**EF**	**GH**	**DE**	**AC**
Jaggery	50g	50g	50g	50g	50g
Peanuts	50g	25g	25g	_	_
Pumpkin seeds	_	25g	_	_	50g
Flax seeds	_	_	25g	50g	_

Table 6.1 – Incorporation of flax seeds and pumpkin seeds in chikki

Where BD: (control), GH: (flax seeds and peanuts), DE: (flax seeds), AC: (pumpkin seeds), EF: (pumpkin seeds and peanuts).

6.5 Organoleptic evaluation

Sensory evaluation is a scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses (sight, smell, taste, touch and hearing) for the purpose of evaluating consumer products. The discipline requires panels of human assessors, on whom the products are tested, and recording the responses made by them. Product is analyzed on the basis of many attributes such as appearance color, flavor (odor and taste), mouth feel, consistency, texture etc.. By applying statistical techniques to the results it is possible to make interferences and insights about the products under test.

Using a 9 point hedonic scale the prepared products were accordingly evaluated organoleptically by the 10 non-trained panelists .One end corresponded to the qualification ‘disliked extremely’ the center to ‘neither liked nor disliked’ and the other end to ‘liked extremely’. The parameters on which the prepared products evaluated were color, taste, flavor, texture and overall acceptability. Total five samples were prepared and presented for organoleptic evaluation.

To assess the quality, acceptability, the product were presented to a panel of ten judges and the evaluation for sensory parameters such as color, taste, flavour, texture and overall acceptability characteristics were carried out using a 9 point hedonic scale (Land and Shepherd ,1988).

Where **AC**- (pumpkin seeds), **BD**- (control), **EF**- (pumpkin seeds and peanuts), **GH**- (flax seeds and peanuts) **DE**- (flax seeds).

6.6 Biochemical analysis

Biochemical constituents and nutritional attributes *viz*. moisture, protein, crude fat, crude fibre, ash and peroxide value, carbohydrate and total energy value were estimated by following standard procedures described as under:

6.6.1 Moisture content (AOAC, 2010)

Moisture content was determined by following the oven drying method. 5g of each sample was taken in a previously weighed, dried aluminium moisture cups. These cups were kept in a hot air oven at 60(±5ºC) for 8 hrs. The aluminium cups were taken out from oven and kept in desiccator for cooling for 30 min, for attaining a constant weight. After cooling, samples were weighed with aluminium cups. The loss in weight represented the moisture content of the sample.

6.6.2 Total ash (AOAC, 2010)

1g of the sample was taken and put in previously dried and weighed silica crucibles. Samples were first incinerated over an electric hot plate followed by ashing in muffle furnace at a temperature of 550(±25ºC) for 6 hrs (until a pale white residue was obtained).These ashed samples were taken out from the muffle furnace and kept in desiccator for 2 hrs for cooling. After cooling samples were weighed again and per cent ash content was calculated.

6.6.3 Crude fibre (AOAC, 2010)

Moisture and fat free sample (2g) were digested with 200ml of 1.25 per cent H_2SO_4 by gentle boiling for half an hour. The contents were filtered and the residue was washed

several times with hot distilled water till it became free from acid. Acid free residue was then transferred to the same flask to which 200ml of 1.25 per cent NaOH was added. The contents were digested again for half an hour, filtered it and residue was again washed with hot distilled till it became alkali free. The residue was dried in an oven overnight at 100ºC and weighed and then placed in muffle furnace at 600ºC (±50ºC) for 4 hours. The loss in weight after ignition the sample represented the fibre in the sample. The per cent crude fibre was calculated as follows:

6.6.4 Crude protein (S. Sadasivam and A. Manickam,2007)

The principle of this method was based on the facts that the Folin-Ciocalteu regents reacts with aromatic residues of proteins and yields blue color which in turn is read in colorimeter. The different proteins contained different aromatic residues. Blue color developed because the alkaline copper reacted with proteins; tyrosin and tryptophan present in protein reduce phosphomolybdate. (present in Folin-Ciocalteu reagent).

Different dilutions of BSA solutions were prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube . The final volume in each of the test tubes was 5 ml. The BSA 23 range was 0.05 to 1 mg/ ml. From those different dilutions, 0.2 ml of protein solution was diluted to different test tubes and 2 ml of alkaline copper sulphate reagent was added(analytical reagent). The solutions were mixed well. The solution was incubated at room temperature for 10 mins. Then 0.2 ml of reagent Folin Ciocalteau solution (reagent solutions) was added to each tube and incubated for 30 min. The readings were taken at 660 nm.

6.6.5 Crude fat (AOAC, 2010)

Fat content was determined by Soxhlet extraction method. Dried samples (2g) were extracted with Petroleum ether in Soxhlet extraction apparatus for 6-8 hours in pre weighed round bottom flask. The extract containing fat and petroleum ether was evaporated over boiling water bath and dried in an oven at low temperature and weighed. The differences in the weight of the round bottom flask represented the ether extract (fat content) present in the sample.

6.6.6 Peroxide value (S. Sadasivam and A. Manickam,2007)

Peroxide value is a measure of the peroxides contained in the oil. The peroxides present are determined by titration against thiosulphate in the presence of potassium iodide. Starch is used as a indicator.

5gm of sample (oil) was taken into a clean dry testing tube and 1gm of powdered potassium iodide and 20ml of solvent mixture was added. Then the tubes were placed in boiling water for 30 seconds but not more the that. Then the contents were transferred to a conical flask containing 20ml of 5% potassium iodide solution. The test tubes were then washed twice with 25ml water each time and were collected into conical flask. It was titrated against N/500 sodium thiosulfate solution until the yellow colour was disappeared. Further 0.5ml starch solution was added and titrated until the blue colour disappears. A blank should also be set at the same time.

6.6.7 Carbohydrate (AOAC 2000)

Carbohydrate estimation was done by using the formula given below:

%Carbohydrate= 100-[moisture+ash+protein+fat].

6.6.8 Total energy value (AOAC 2000)

Energy value was calculated by using the undermentioned formula:

Energy= [(9×g.fat)+(4×g.protein)+(4×g.carbohydrate)]

6.7 Storage study

The shelf life of food is the time period within which the food to be consumed is safe and is of acceptable quality to consumers. The life of food product depends upon various factors such as storage conditions, temperature etc. Therefore in this particular investigation, after the preparation of flax seeds and pumpkin seeds chikki ,they were kept for storage in air tight containers (plastic) at room temperature for 30 days within the campus of Lovely Professional University, Punjab**.** All the samples were then analyzed on the basis of texture and were organolepticaly evaluated by ten panelists using 9 point hedonic scale.

6.8 Statistical Analysis

The experiments were carried out in triplicate and the data so obtained was subjected to average and standard difference between the different samples was calculated and were documented.

6.9 Expected outcome

If flax seeds and pumpkin seeds 'chikki' developed, is consumed in the desired amounts i.e 20-25 gms per day by hypercholesterolemic patients, these will be beneficial for them, as it would maintain the acceptable levels of cholesterol and other fats in the body thereby preventing any further complications. Also if consumed by normal population as a snack, it can help to prevent any future physiological condition related to any high cholesterol intake.

CHAPTER -7

RESULTS AND DISCUSSION

Any research work is done in order to approach a query and to march ahead on the road of progress and development. Result of research done thus contributes to the final step, through which the major desired objective can be attained.

This study was undertaken in an attempt to develop some indigenous recipes with flax seeds and pumpkin seeds by keeping hypercholesterolemic patients in mind, and followed by its chemical analysis. The research work was carried in the laboratory of Department of Food Technology and Nutrition at Lovely Professional University, Punjab. The purpose of developing these value added products was to provide adaptable, acceptable and nutritive recipe for hypercholesterolemic patients and also for the community.

The study was based on the various objectives which were focused during the research work and are discussed below:

1) Development of quality products for hypercholestrolemic patients using flax seeds and pumpkin seeds.

2) Organoleptic evaluation of the developed products.

3) Nutritional evaluation of the developed products.

4) Shelf life study of the products.

7.1 DEHYDRATION OF FLAX AND PUMPKIN SEEDS:

Flax and pumpkin seeds were dehusked, washed and dried by oven drying.

7.1.1Time taken in dehydration

The result of time taken in dehydration by the oven drying is given in table below:

Types of seeds	Time taken in hours	Technique	Temperature
Flax seeds	90 minutes	Oven drying	60ºC
Pumpkin seeds	120 minutes	Oven drying	60ºC

Table 7.1: Time taken for dehydration of seeds

Pumpkin seeds took more time for drying then flax seeds . Oven drying is very efficient method but becomes difficult to be preached in the community as it is an expensive method of drying and the community cannot afford ovens for drying the seeds therefore sun drying can also be opted (Table 7.1).

7.2 ORGANOLEPTIC EVALUATION

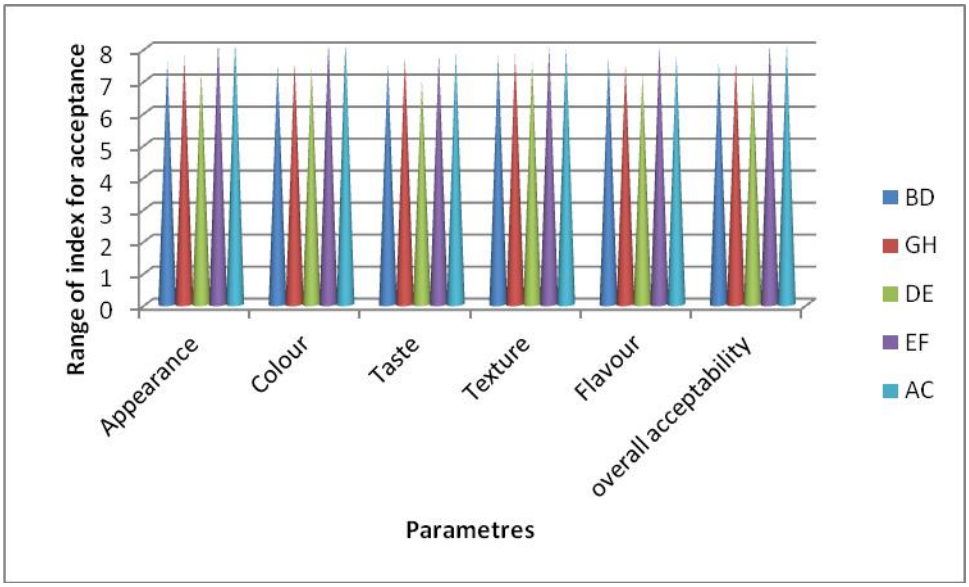
Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (Stone and Sidel, 1993). Sensory methods are the controlled methods which gives results that can be statistically evaluated. Using a 9 point hedonic scale the prepared products were accordingly evaluated organoleptically by the 10 non-trained panelists In the hedonic scale one end corresponded to the qualification was ‘disliked extremely’ the center to ‘neither liked nor disliked’ and the other end to ‘liked extremely’. The parameters on which the prepared products evaluated were color, taste, flavor, texture, appearance and overall acceptability .

Fig: 7.1 Flaxseed and Pumpkin seed Chikki

Parameters	BD	GH	DE	EF	AC
Appearance	7.6± 0.69	7.75± 0.63	7.3± 0.67	8.3± 0.48	8.3± 0.48
Colour	7.5± 0.70	7.55± 0.68	7.4± 0.69	8.3± 0.48	8.3± 0.48
Taste	7.5± 0.91	7.7±0.94	7± 1.41	7.8± 0.78	7.9±0.73
Texture	7.8± 0.78	7.8± 0.91	7.6± 1.07	8.1± 0.56	8.1± 0.56
Flavor	7.7±0.67	7.5±0.84	7.2±1.54	8.1±0.56	7.8±0.42
Overall acceptabilty	7.57±0.64	7.6±0.72	7.26±0.86	8.2±0.47	8.15±0.50

Table 7.2: Sensory evaluation of the food samples .

Mean ± standard deviation (n=10)



Graph : 7.1 Index of acceptance of the products

Where **AC**- (pumpkin seeds), **BD**- (control), **EF**- (pumpkin seeds and peanuts), **GH**- (flax seeds and peanuts) **DE**- (flax seeds).

Appearance

The Data obtained in Table 4 indicated that the product AC and EF had obtained the highest mean score for appearance (8.3). The mean score for other samples of chikki was ranging between 8 and 9. The scores revealed that all the products were falling in the range of "liked very much" and "liked extremely" categories. The least acceptability was of DE. The increasing colour of the chikki was attributed to the increasing flax seeds content and hence DE with highest flax seeds content was least acceptable.(Table 7.2)

Color

According to the data obtained the mean score for color was attained by the product AC and EF (8.3), closely followed by BD and GH both obtaining score (7.5 and 7.55) and least acceptable was DE (7.4). The color of the sample DE was not much acceptable to the panels. All the samples were ranging between 8 and 9. And the results revealed that all the products were in the range of "liked very much" to "liked extremely". (Table 7.2)

Texture

Table 4 clearly depicted that the maximum number of panel members scored product AC and EF (both scoring 8.1) as the best samples. Second best was BD and GH (7.8) and least acceptable was DE (7.6) because the increasing content of flax seeds might have decreased the crispness and brittleness of the product. (Table 7.2)

Taste

The mean score for taste was found to be ranging from 7 to 8 which indicated that all the samples were ranging from "liked moderately" to "liked very much" category. The highest score was obtained by the product AC and EF (7.9 and 7.8), followed by GH (7.7) and then BD and DE i.e. 7.5 and 7 respectively. The difference in score was due to altering changes in tastes due to different concentrations of the seeds incorporated. The increased content of flax seeds might have imparted the bitter taste, hence the product which was made only using flax seeds was least acceptable. (Table 7.2)

Flavor

The mean score for flavor were found to be ranging from 7.2 to 8.1. The highest score was obtained by EF (8.1) closely followed by AC (7.8). The score was then followed by BD and GH i.e. 7.7 and 7.5 respectively. This data predicted that all products were falling in the range of "liked moderately" and "liked very much" category. The scores for flavor were affected by the content of flax seeds in the products, and the one with highest incorporation of flax seeds scored least marks. (Table 7.2)

Overall acceptability

The profile for overall acceptability of products developed during present study ranged from 7 to 9. None of the products were totally disliked by the judges, all were falling in the category of "liked moderately" to "liked extremely." (Table 7.2)

Mean score

The mean score was represented by calculating the mean of all sensory characteristics of the products. Table 7.2 extrapolates highest mean score for every characteristic for sample EF

(pumpkin seeds and peanuts) and thus was considered the best among all. Second best was found to be sample AC (pumpkin seeds) . The lowest mean score obtained by DE (flax seeds) was least acceptable.

It was concluded that among all samples none of the sample was completely disliked and were all acceptable by the panelists. The sample DE (flax seeds) showed little decline in scores. This may be accounted to the effects of peculiar taste of flax seeds and flavour as well as appearance of the same. (Table 7.2)

7.3 Biochemical estimations of the products developed

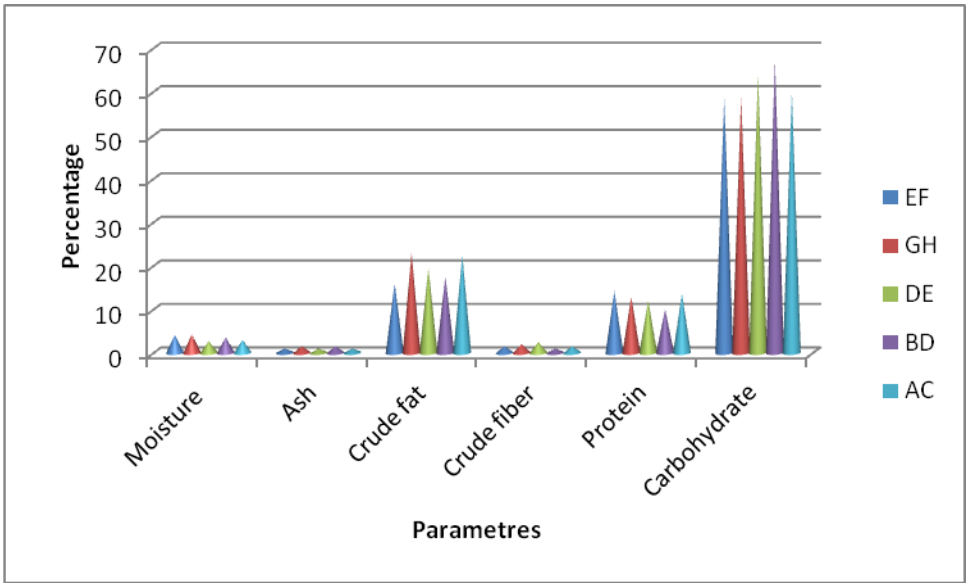
Product involving incorporation of flax seeds and pumpkin seeds were finally tested biochemically using the methods given by AOAC, 2010.

Biochemical estimations of nutrients present in the prepared product (chikki) were done. These were analyzed for crude fat, crude fibre, moisture, ash, protein and peroxide value etc. Table 5 shows the estimated values of various nutrients per 100g of chikki.

Parameters	BD	GH	DE	EF	AC
Moisture	3.6± 0.02	3.8± 0.04	3.4± 0.06	4.2±0.005	3± 0.01
Ash	1.5± 0.01	1.5± 0.01	1± 0.005	1± 0.01	1± 0.005
Crude fat	17.5± 0.10	23± 0.03	19.5± 0.08	22 ±0.05	22.5±0.03
Crude fiber	1.6± 0.01	2.3± 0.01	2.6± 0.56	2± 0.005	2± 0.005
Protein	10.1± 0.05	12.92±0.08	12.06± 0.14	14.2± 0.15	13.6± 0.07
Carbohydrate	67.3	58.78	64.04	58.6	59.9
Total Energy	467.1kcal	493.8 kcal	479.9 kcal	489.2kcal	496.5 kcal

Table 7.3: Nutritional evaluation of formulated products (%)

Mean ± standard deviation(n=3)



Graph: 7.2 Nutritional content of the food samples

Where **AC**- (pumpkin seeds), **BD**- (control), **EF**- (pumpkin seeds and peanuts), **GH**- (flax seeds and peanuts) **DE**- (flax seeds).

Moisture content

The moisture content in the five samples of chikki which had undergone oven drying was in the range of 3- 4.2%. The moisture content in the sample EF was highest i.e 4.2%. This was followed by the sample GH, DE and BD with respective moisture content i.e (3.8%,3.6% and 3.4%) . The sample which consisted the least moisture content was AC i.e (3%). The highest mean value obtained for sample EF- 4.2% was less than other values reported in literature, i.e 5% (Elinge C.M *et al* 2012).The lower moisture content of the seed will give it a storage advantage. (Table 7.3).

Ash content

The ash content in the five samples was in the range of 1-1.5%. The sample having ash content highest amongst all was BD and GH(1.5%). And the samples having least ash content were AC, EF and DE i.e (1%) respectively. The highest mean value obtained for sample BD and GH (1.5%) was less than other values reported, i.e 3.4%. and Ash is the mineral-rich residue left after samples are burned (Morris D.H, 2003). (Table 7.3)

Crude Fiber content

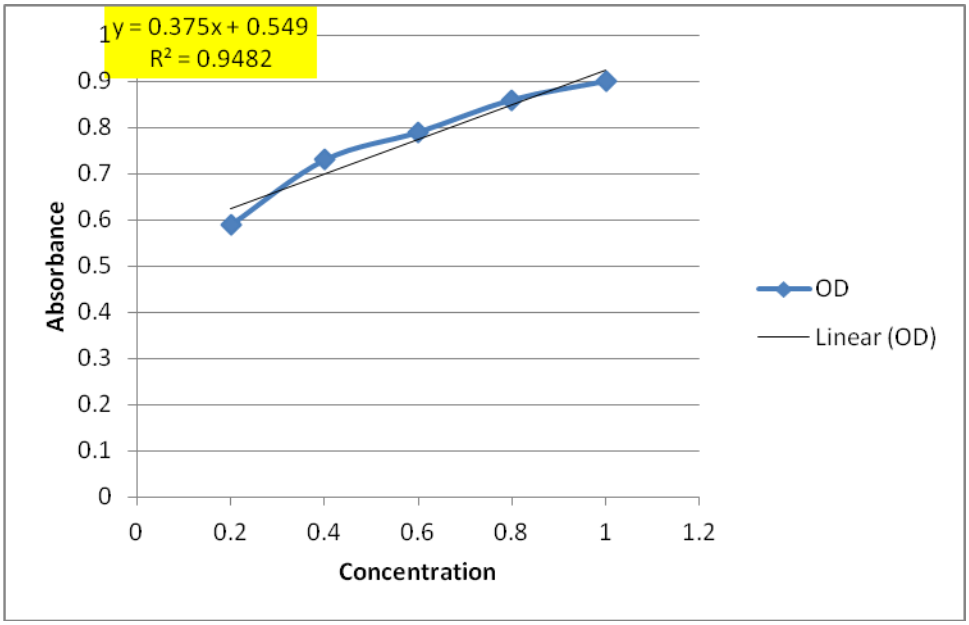
The fiber content in the five samples was in the range of 1.6 – 2.6%, with the highest level in the sample DE (2.6%) which was less then as reported by (Mervat E.D, 2015) as 3.01% , which may be reduced while roasting and cooking, followed by sample GH which was (2.3%) , and sample EF and AC had (2%) and the minimum values in the sample BD which came out to be (1.6%). (Table 7.3)

Crude fat content

The fat content in the five samples was in the range 16 – 23%. The sample contributing to the highest fat content was GH (23%) which was less than the value reported in review of literature (41%). The composition of flaxseed can vary with genetics, growing environment, seed processing and method of analysis. (Daun *et al*., 2003). The samples having second maximum fat content was DE, AC and EF (19.5%, 22.5% and 22%). The sample possessing least fat content was BD i.e (17.5%). (Table 7.3)

Protein

The protein content in the five samples was in the range 10.1%-14.2%. The sample contributing to the highest protein content was EF (14.2%) which was relatively less as reported by (Oluyemi, E.A , 2006) i.e 27.48% , but can be served as a source of protein considering the level of protein deficiency in the society. Flaxseed is a source of good-quality protein and albumins and globulins are the storage proteins of flaxseed with globulins forming the highest portion (58-66% of the total seed protein) which may be reduced due to cooking (Chung, Lei, & Li-Chan, 2005). The samples having second maximum protein content was DE, AC and GH (12.06%, 12.92% and 13.6%). The sample possessing least protein content was BD i.e (10.1%). (Table 7.3)



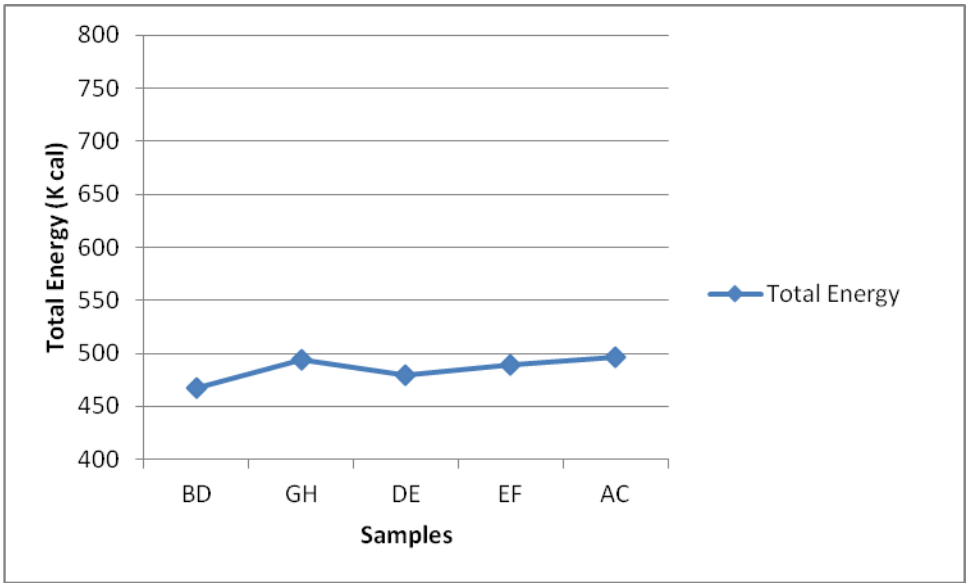
Graph 7.3: Standard curve for protein

Carbohydrate

The content of carbohydrate in five samples which were calculated came out to be in range 58.6%- 67.3%. The sample possessing the highest carbohydrate content was BD (59.9%). The samples having second maximum carbohydrate content was DE, AC and GH (64.74%, 59.9% and 58.88%). which were relatively higher than the values reported by (Morris D.H, 2003) as 29% and can be considered as a good source of carbohydrates because of the addition of peanuts also. The sample possessing least carbohydrate content was EF i.e (58.6%). (Table 7.3)

Total Energy

In terms of energy all the five samples came out to be in range 467.1 kcal- 596.5 kcal. The sample possessing the highest energy content was AC (532.9kcal). The samples having second maximum energy content was GH ,EF and DE (493.8kcal, 489.2kcal and 479.9 kcal respectively. The sample possessing least total energy content was BD i.e (467.1 kcal). (Table 7.3)



Graph : 7.4 Total energy content of the food samples

Where **AC**- (pumpkin seeds), **BD**- (control), **EF**- (pumpkin seeds and peanuts), **GH**- (flax seeds and peanuts) **DE**- (flax seeds).

Peroxide value:

Peroxide value is relatively a measure of the peroxides content in the oil. Peroxide value thus gives an indication about the rancidity of a sample. In this particular study peroxide value was determined between 1-2 days after preparation of samples. Therefore in this experiment conducted resulting peroxide value of five samples which were tested ranged between 2-4 meq/kg. The highest peroxide value was found to be in sample GH (4 meq/kg). Following it the samples having less peroxide value then this were AC, EF and DE (3.8, 3.2 and 2.86 meq/kg) respectively.

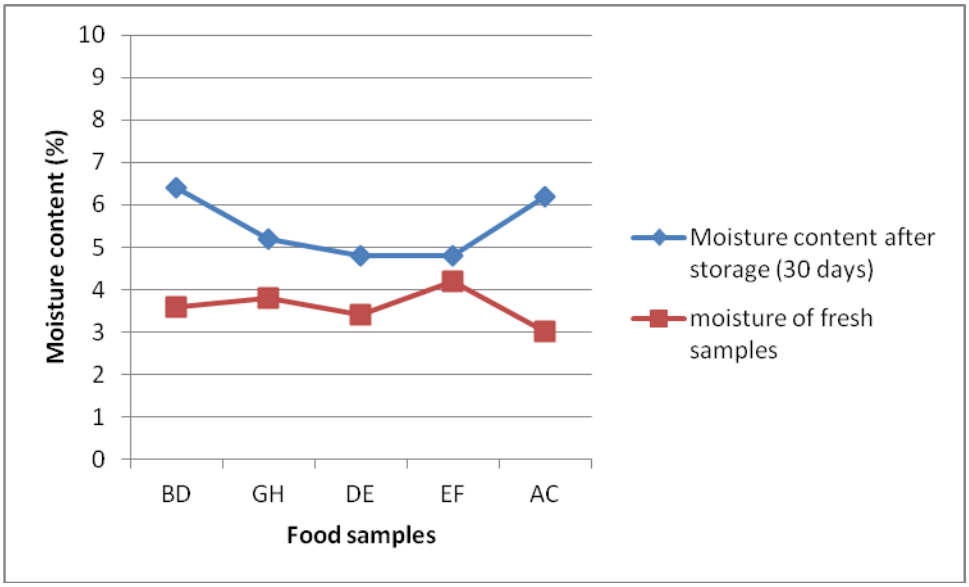
The sample having least peroxide value was BD (2.1 meq/kg). Therefore it was interpretated that the peroxide value was not increased because of the estimation done on freshly prepared samples.

Where **AC**- (pumpkin seeds), **BD**- (control), **EF**- (pumpkin seeds and peanuts), **GH**- (flax seeds and peanuts) **DE**- (flax seeds).

7.4 Storage studies

After keeping the samples at room temperature (38ºC) in air tight container for 30 days, the samples were tested for moisture content.

Moisture content: The moisture content was found to be increased as compared to the samples which were freshly prepared and the increase was seen as 6.2% in sample AC, 4.8% in sample EF, 6.4% in sample BD, 4.8% in sample DE and 5.2% in sample GH approximately Thus freshly prepared samples were more acceptable.

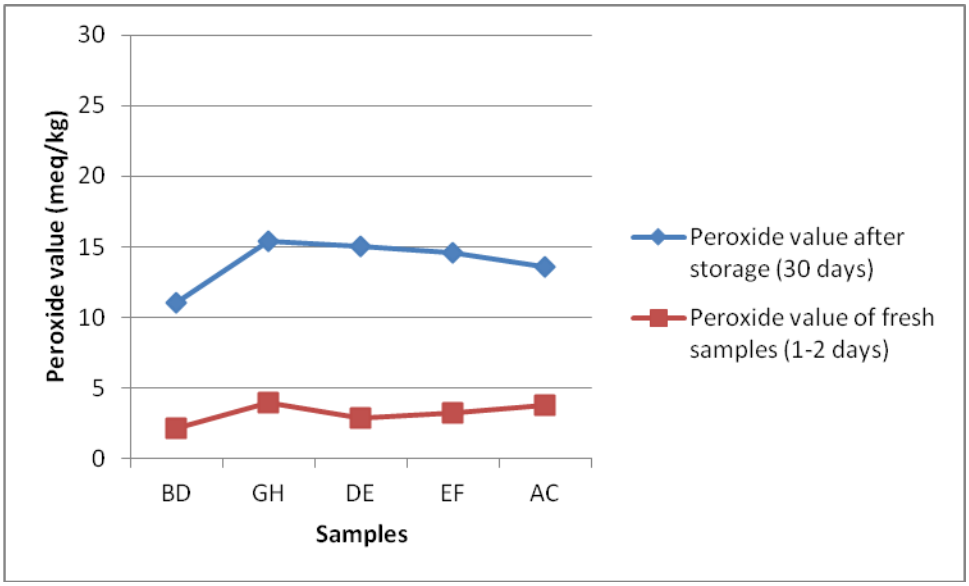


Graph : 7.5 Moisture content after and before storage of food samples

Peroxide value: Peroxide value is relatively a measure of the peroxides content in the oil. Peroxide value thus gives an indication about the rancidity of a sample. Therefore in this experiment conducted resulting peroxide value of five samples which were tested after storage for 30 days ranged between 11-15.4 meq/kg. The highest peroxide value was found to be in sample GH (15.4 meq/kg). Following it the samples having less peroxide value then this were AC, EF and DE (15, 14.6 and 13.6 meq/kg) respectively.

The sample having least peroxide value was BD (11 meq/kg). Therefore it was interpretated that as the concentration of flax seeds and pumpkin seeds increased in the product which was tested

the peroxide value also increased which meant that there was an increase of rancidity in the samples.



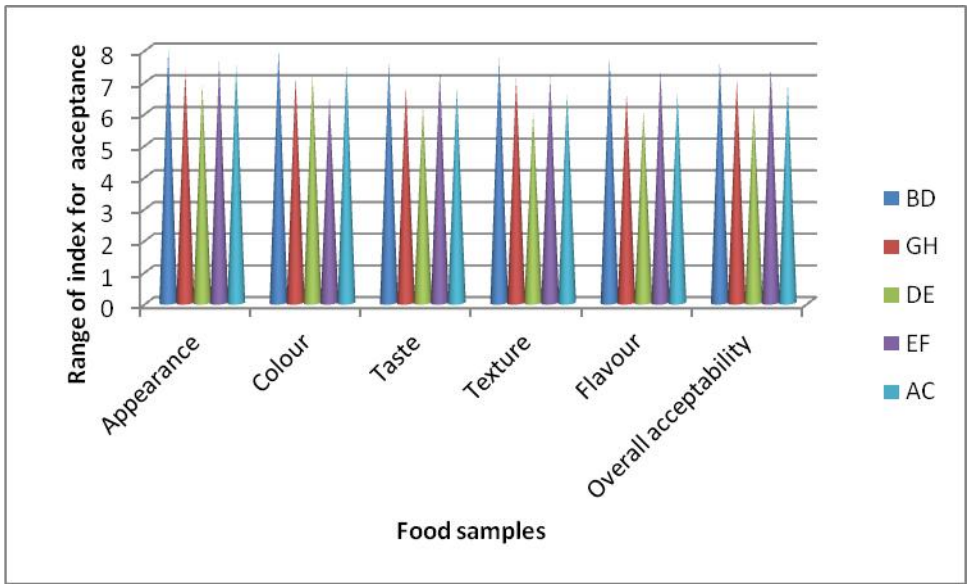
Graph : 7.6 Peroxide value after and before storage of food samples

Organoleptic evaluation: After the storage of samples for 30 days they were sensory evaluated by ten panelists using 9 point hedonic scale. The products were withdrawn after 30 days and the quality and stability of the samples was evaluated by estimating the moisture content and sensory evaluation. As a result, the samples evaluated after the storage study were less acceptable as compared to the freshly prepared samples. The major variation noticed was the changes in texture in almost all the samples kept for storage study. The changes were seen as decrease in crispness and brittleness of the product which might be due to exposure to moisture.

Parameters	BD	GH	DE	EF	AC
Appearance	8± 0.66	7.3± 1.33	6.9± 1.44	7.6± 0.84	7.5±1.17
Colour	8± 0.66	7.1± 1.28	7.2± 0.78	6.5± 1.35	7.5± 0.97
Taste	7.6± 0.84	6.8±1.03	6.2± 2.04	7.3± 1.25	6.8±1.54
Texture	7.75± 0.63	7.1± 1.66	6± 1.94	7.15± 1.63	6.6± 1.71
Flavor	7.7±0.67	6.6±0.96	6.05±2.31	7.35±1.20	6.6±1.42
Overall acceptability	7.68±0.87	7.03±1.301	6.23±1.85	7.41± 1.14	6.9±1.37

Table 7.4: Sensory evaluation of the food samples after storage

Mean ± standard deviation (n=10)



Graph 7.7: Index of acceptance of the products after storage study.

Where **AC**- (pumpkin seeds), **BD**- (control), **EF**- (pumpkin seeds and peanuts), **GH**- (flax seeds and peanuts) **DE**- (flax seeds).

Appearance

The Data obtained in Table 7 clearly indicated that the product BD had obtained the highest mean score for appearance (8). The mean score for other samples of chikki was ranging between 6.9 and 7.6. The scores revealed that all the products were falling in the range of "liked slightly" and "liked moderately" categories. The least acceptability was of DE. The increasing concentration of flax seeds lead to little non appealing appearance and hence DE with highest flax seeds content was least acceptable. (Table 7.4)

Color

According to the data obtained the mean score for color was attained by the product BD (8), closely followed by AC and DE both obtaining score (7.5 and 7.2) and least acceptable was EF (6.5). The color of the sample EF was not much acceptable to the panels. All the samples were ranging between 6.5 and 8. And the results revealed that all the products were in the range of "liked slightly" to "liked very much". (Table 7.4)

Texture

Table 7.4 clearly depicted that the maximum number of panel members scored product BD (7.75) as the best sample. Second best was EF and GH (7.15, 7.1) and least acceptable was DE and AC(6.6, 6) because the increasing content of flax seeds might have decreased the crispness and brittleness of the product. (Table 7.4)

Taste

The mean score for taste was found to be ranging from 6.2 to 7.6 which indicated that all the samples were ranging from "liked slightly" to "liked moderately" category. The highest score was obtained by the product BD and EF (7.6 and 7.3), followed by GH and AC (6.8) respectively. The least acceptable was DE (6.2). The difference in score was due to altering changes in tastes due to different concentrations of the seeds incorporated. The increased content of flax seeds might have imparted the bitter taste, hence the product which was made only using flax seeds was least acceptable. (Table 7.4)

Flavor

The mean score for flavor were found to be ranging from 6.6 to 7.7. The highest score was obtained by BD (7.7) closely followed by EF (7.35). The score was then followed by AC and GH i.e. (6.6) respectively. This data predicted that all products were falling in the range of "liked

slightly" and "liked moderately" category. The scores for flavor were affected by the content of flax seeds in the products, and the one with highest incorporation of flax seeds scored least marks i.e DE (6.05). (Table 7.4)

Overall acceptability

The profile for overall acceptability of products developed during present study ranged from 6 to 8. None of the products were totally disliked by the judges, but reported about the variation in texture of the samples. All samples were falling in the category of "liked slightly" to "liked very much." (Table 7.4)

Mean score

The mean score was represented by calculating the mean of all sensory characteristics of the products. Table 7.4 extrapolates highest mean score for every characteristic for sample BD (control) and thus was considered the best amongst all. Second best was found to be sample EF (pumpkin seeds and peanuts). The lowest mean score obtained by DE (flax seeds) was least acceptable. (Table 7.4)

It was concluded that among all samples none of the sample was completely disliked and were all acceptable by the panelists. The sample DE (flax seeds) showed little decline in scores. This may be accounted to the effects of peculiar taste of flax seeds and texture as well as appearance of the same. (Table 7.4)

CHAPTER- 8

CONCLUSION AND FUTURE SCOPE

Cardiovascular diseases are serious and widespread public health problems. Their global scale and magnitude, combined with their damaging physiological socioeconomic effects, require the urgent adoption of known and effective measures to tackle this critical problem. With the knowledge that the intake of foods rich in fiber ,antioxidants and various other nutrients reduce the prevalence of hypercholesterolemia significantly, much focus had been placed on fortification rather than on increasing food consumption and improving and diversifying diets. However, in practice many of the programmes being set up proved difficult to be managed, more costly than expected to implement, and less effective than promised. As these programmes have had little reported success in reducing hypercholesterolemia, interest is turning to food-based approaches that have higher potential for achieving far-reaching and long-lasting benefits for the control of hypercholesterolemia. Food-based approaches aim to improve nutrition through increasing the availability and consumption of a nutritionally adequate and micronutrient rich diet made up from a variety of available foods. Food based approaches are recognized as an essential part of an urgently needed more comprehensive strategy to combat such diseased conditions. There are a number of actions that may be taken by international agencies, governments, line ministries of agriculture, health, education, industry and the private sector, communities and households themselves that are feasible and practical and that will increase the consumption of nutritionally adequate diets. Food-based strategies aim to improve the quality of the overall diet by increasing the availability and consumption of a wider range of foods.

Seeds are a rich source of macronutrients and micronutrients. But besides its abundant health effects they are being wasted due to lack of knowledge, processing and preservation infrastructure.

In the present study also, Flax seeds and Pumpkin seeds which are a good source of nutrients was researched on with the following objectives:

1) Development of quality products for hypercholestrolemic patients using flax seeds and pumpkin seeds.

2) Organoleptic evaluation of the developed products.

3) Nutritional evaluation of the developed products.

4) Shelf life study of the products.

The products were developed by Incorporating Flax seeds and Pumpkin seeds in the basic recipe, in different concentrations which were as given: Flax seeds- 50% and 25% and Pumpkin seeds- 50% and 25% respectively. And the acceptability of these were checked by a panel of 10 members on the basis of various attributes like taste, appearance, texture, color, flavour and overall acceptability of the products with 9 point Hedonic Scale. Also the samples prepared were kept for storage study for 30 days and were organolepticaly evaluated

Thus the biochemical estimation of formulated products showed the following results: Moisture content was highest in EF and GH(4.2% , 3.8%). Ash content was highest in EF and GH (1%, 1.5%). Crude fat was highest in GH- 23%. Crude Fiber was highest in the DE (2.6%). Protein content was highest in EF-(14.2%) .Carbohydrate was highest in BD-67.3.% and Total energy content was highest in AC-496.5 kcal. The least retention was seen in the following samples: Moisture- 3% in AC ,Ash- 1% in EF, DE and AC, Crude fat- 16% in EF, Crude fiber- 1% in BD ,Protein -10.1% in BD, Carbohydrate- 58.6% in EF and Total energy-467.1 in BD.

The difference in the values obtained in current study with those reported in literature may probably be due to the impact of several factors as varietal differences, maturity levels, environmental conditions, cooking temperatures, storage conditions, etc.

Salient findings of the study

- The Flax seeds and Pumpkin seeds took approximately 90- 120 minutes for complete drying and were as such incorporated in the product.
- Maximum amount of nutrient retention was found in different samples.
- The formulated products were overall acceptable.
- The acceptability of the product decreased with the increase in the level of the Flax seeds.
- The nutrient content in the incorporated recipes increased with the increase in the level of Flax seeds and Pumpkin seeds.

Flax seeds and Pumpkin seeds were acceptable in raw form and could be incorporated in convectional food items to improve the nutritional quality of the product as well as add variety in the diet. Roasting of flax seeds and Pumpkin seeds helped by making them suitable to be used in the products to enhance its flavor and taste and can be used as a product for Hypercholesterolemic patients. Thus the Flax seeds and Pumpkin seeds can be used as a natural fortifier and can be incorporated in different products.

Work in pursuit of this strategy includes continuing efforts to ensure that dietary diversification, food fortification, supplementation, and public health measures are taken comprehensively to combat Hypercholesterolemia. Many aspects that still need to be considered and investigated are given below and can be considered for future research:

- To develop a quick and single reliable methods of analysis.
- Increase the consumption of nutrient rich foods that meet dietary needs and food preferences.
- Explore ways to increase financial investments in food-based initiatives at the country level including by better quantifying the contribution that such interventions can make to demonstrate their efficacy.
- A list of best practices can be drawn that households can adopt to prevent Hypercholesterolemia.

CHAPTER- 9

REFERENCES

1. Stapleton et al. Journal of Inflammation. Hypercholesterolemia and microvascular dysfunction: interventional strategies. 2010; 7:54.
2. Biggerstaff KD, Wooten JS . Understanding lipoproteins as transporters of cholesterol and other lipids. Adv Physiol Educ. 2004; 28 (1–4): 105–6.
3. Carmena R, Duriez P, Fruchart JC . Atherogenic lipoprotein particles in atherosclerosis. Circulation. 2004; 109 (23 Suppl 1): III2–7.
4. Durrington, P . Dyslipidaemia. The Lancet .2003;362 (9385): 717.
5. Hooper L, Summerbell CD, Thompson R, et al. Reduced or modified dietary fat for preventing cardiovascular disease. In Hooper, Lee. Cochrane Database Syst Rev. (2012).
6. Prasad K, Singha K K. Prevelance of hypercholesterolemia in young Indians. 2010
7. Bhatnagar D, Soran H, Durrington PN . Hypercholesterolaemia and its management. 2008; BMJ 337: a993.
8. Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R Concept of vulnerable/unstable plaque. Arterioscler. Thromb. Vasc. Biol. 2010. 30 (7): 1282–92.
9. Grundy, SM; Balady, GJ; Criqui, MH; Fletcher, G; Greenland, P; Hiratzka, LF; Houston-Miller, N; Kris-Etherton, P; Krumholz, HM; Larosa, J.; Ockene, I. S.; Pearson, T. A.; Reed, J.; Washington, R.; Smith, S. C. . Primary prevention of coronary heart disease: guidance from Framingham: a statement for healthcare professionals from the AHA Task Force on Risk Reduction. American Heart Association". Circulation 1998; 97 (18): 1876–87…[Durrington, P . Dyslipidaemia. The Lancet .2003;362 (9385): 717.]
10. Howell WH, McNamara DJ, Tosca MA, Smith BT, Gaines JA . Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. Am. J. Clin. Nutr. 1997; 65 (6): 1747–64.
11. Lichtenstein AH . Intestinal cholesterol metabolism. Ann. Med. 1990;22 (1): 49–52.
12. Sacks FM, Katan M . Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. Am. J. Med. 2002; 113 Suppl 9B: 13S–24S

13. Schaefer, EJ; Gleason, JA; Dansinger, ML . Dietary fructose and glucose differentially affect lipid and glucose homeostasis" .The Journal of nutrition 2009; 139 (6): 1257S–1262S
14. Tang JL, Armitage JM, Lancaster T, Silagy CA, Fowler GH, Neil HA . Systematic review of dietary intervention trials to lower blood total cholesterol in free-living subjects". BMJ 1998;316 (7139): 1213–20.
15. National Institute for Health and Clinical Excellence. Clinical guideline 67: Lipid modification. London, 2008.
16. Ray et al; Seshasai, S. R. K.; Erqou, S.; Sever, P.; Jukema, J. W.; Ford, I.; Sattar, N. "Statins and All-Cause Mortality in High-Risk Primary Prevention: A Meta-analysis of 11 Randomized Controlled Trials Involving 65 229 Participants". Arch Intern Med 2010; 170 (12): 1024–31.
17. Madhusudhan B. Potential Benefits of Flaxseed in Health and Disease - A Perspective. Agriculturae Conspectus Scientificus (ACS) .2009 ; 74 : 2 : 67-72.
18. Xanthopoulou MN, Nomikos T, Fragopoulou E et al. Antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts. Food Research International. 2009 ;42, (5—6), 641-646.
19. Stapleton PA, Goodwill AG, James ME, Frisbee JC: Altered mechanisms of endothelium-dependent dilation in skeletal muscle arterioles with genetic.2010
20. Hypercholesterolemia. Am J Physiol Regul Integr Comp Physiol 2007, 293:R1110-R1119
21. See, E.F., Wan Nadiah, W.A. Noor Aziah, A.A. ASEAN Food Journal Physico-Chemical and Sensory Evaluation of Breads Supplemented with Pumpkin Flour.2007; 14 (2): 12 3 - 1 3 0
22. Soons KR, Little DN, Harvey J. Cholesterol screening in the elderly: changing attitudes. Gerontology .1995;41:57-62
23. Manolio TA, Furberg CD, Wahl PW, et al. Eligibility for cholesterol referral in communitydwelling older adults. The Cardiovascular Health Study. Ann Intern Med 1992;116:641-9.
24. Lloyd-Jones D, Adams R, Carnethon M, De SG, Ferguson TB, Flegal K, Ford E, Furie K, Go A, Greenlund K, et al.: Heart disease and stroke statistics update.a report from the

American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2009, 119:e21-181.

25. Stapleton PA, Goodwill AG, James ME, D'Audiffret AC, Frisbee JC: Differential impact of familial hypercholesterolemia and combined hyperlipidemia on vascular wall and network remodeling in mice. Microcirculation 2010, 17:47-58.
26. Costa J, Borges M, Oliveira E, Gouveia M, Carneiro AV. Incidence and prevalence of hypercholesterolemia in Portugal: a systemic review. 2013.
27. Bandana S. Diet and lifestyle and its association with cholesterol. International Journal of Medicine and Biomedical Research. 2012; 1 (2).
28. Dewan BD, Malhotra KC, Gupta SP.Epidemiological study of coronary heart disease in rural community in Haryana.Indian Heart J. 1974 Apr;26(2):68-78.
29. AM Sawant, Dhanashri Shetty, R Mankeshwar, Tester F Ashavaid. Prevalence of Dyslipidemia in Young Adult Indian Population. 2010.
30. Ellegard LH, Andersson SW, Normen AL, Andersson HA. Dietary plant sterols and cholesterol metabolism. Nutr Rev. 2007 ;65(1):39-45.
31. Harris KA, Kris-Etherton PM. Effects of whole grains on coronary heart disease risk. Curr. Atheroscler. Rep. (2010).
32. Ullrich IH. Dietary fibre plays a protective role. J Am Coll Nutr. 1987;6(1):19-25.
33. Glew RH, Glew RS, Chuang LT et al. Amino acid, mineral and fatty acid content of pumpkin seeds (Cucurbita spp) and Cyperus esculentus nuts in the Republic of Niger. Plant Foods Hum Nutr. 2006 Jun;61(2):51-6.
34. Pan A., Danxia Yu, Wendy Demark- Wahnefried, Oscar H Franco And Xu Lin. 2013.
35. Meta-Analysis of the Effects Of Flaxseed Interventions On Blood Lipids. Am J Clin Nutr. 2009; 90: 288-297.
36. Simbalista RL, Sauerbronn AV, Aldrighi JM, Areas JA. Consumption of a flaxseed-rich food is not more effective than a placebo in alleviating the climacteric symptoms of postmenopausal women. J Nutr. 2010;140(2):293-7.
37. Dodin S, Lemay A, Jacques H, Legare F, Forest JC, Masse B. The effects of flaxseed dietary supplement on lipid profile, bone mineral density, and symptoms in menopausal women: A randomized, double-blind, wheat germ placebo-controlled clinical trial. J Clin Endocrinol Metab. 2005;90(3):1390-7.

38. Bloedon LT, Balikai S, Chittams J, Cunnane SC, Berlin JA, Rader DJ, et al. Flaxseed and cardiovascular risk factors: Results from a double blind, randomized, controlled clinical trial. J Am Coll Nutr. 2008;27(1):65-74.
39. Arjmandi BH, Khan DA, Shanil Juma, Drum ML, Sreevidya Venkatesh, Sohn E, et al. Whole flaxseed consumption lowers serum LDL-cholesterol and lipoprotein(a) concentrations in postmenopausal women. Nutr Res. 1998;18(7):1203-14.
40. Dupasquier, C.M., Weber, A.M., Ander, B.P., Rampersad, P.P., Steigerwald, S., Wigle, J.T., Mitchell, R.W., Kroeger, E.A., Gilchrist, J.S., Moghadasian, M.M., Lukas, A., and Pierce, G.N. Effects of dietary flaxseed on vascular contractile function and atherosclerosis during prolonged hypercholesterolemia in rabbits. Am J Physiol Heart Circ Physiol 2006.
41. Prasad, K. Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed. Circulation. 1999; 99: 1355-1362.
42. S.Y. Al-Okbi, D.A. Mohamed, E. Kandil, E.K. Ahmed and S.E. Mohammed. Functional ingredients and cardiovascular protective effect of pumpkin seed oils. 2013
43. Abuelgassim, A., Al-Showayman . The Effect of pumpkin (Cucurbitapepo L.) seeds and L- arginine supplementation on serum lipid concentrations in atherogenic rats. AJTCAM. 2012; 9(1):131.
44. Tawheed Amin, Monika Thakur. Cucurbita mixta (pumpkin seeds)- A general overview on their health benefit. 2013; 4:6:846-854.
45. Landekaa I, Dikicb D, Radisica I, Teparica R, Druzzina BV and Rogicc D.: Croatian Journal of Food Technology, Biotechnology and Nutrition. 2011; 6 (1-2), 63-68 .
46. Tripathy B.B. Das S. lipid disorders: implications and management. Indian college of physicians. 2002.
47. He Ka et al. The puzzle of dietary fat intake and risk of ischemic stroke. A brief review of epidemiological data, J Am diet assoc. 2007; 107, 287-295.
48. Kristensen et al. Nutrition & Metabolism .Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depend on food type 2012; 9:8
49. Elinge C. M International Journal of Plant Research. Proximate, Mineral and Anti-nutrient Composition of Pumpkin (*Cucurbitapepo L)* Seeds Extract 2012; 2(5): 146-150

50. Nyam KL , Lau M , Tan CP . Mal J Nutr. Fibre from Pumpkin (*Cucurbita pepo* L.) Seeds and Rinds: Physico-chemical Properties, Antioxidant Capacity and Application as Bakery Product Ingredients.2013;19(1):19-109
51. AOAC (2010). Official Methods of Analysis, 17 TH Ed., Association of Official Analytical Chemists, Washington DC.
52. Tarpila A , Wennberg T , Tarpila S. Flax seeds as a functional food .Current Topics in Neutraceutical Research 2005;Vol. 3, No. 3, pp. 167-188.
53. .Patel S. Pumpkin (Cucurbita sp.) Seeds as nutraceutic: a review on status quo and scopes Mediterr J Nutr Metab, 2013.
54. Mishra A et al. Soluble Dietary Fiber: Clinical Nutrition Uses. Der Pharmacia Lettre, 2010, 2(5): 371-378.
55. (S. Sadasivam, A. Manickam. Biochemical methods, New Age International (P) Limited, 2007.
56. Morris, D.H. Flax: A health and nutrition primer.Winnipeg: Flax Council of Canad.2003., 3, 11.
57. Daun, J.K., Barthet, V.J., Chornick, T.L. and Duguid, S. 2003. Structure, composition, and variety development of flaxseed. In Thompson, L. U. and Cunnane, S. C. (Eds). Flaxseed in Human Nutrition. Champaign, Illinois: AOCS Press, 2003,2:1-40.
58. Oluyemi, E. A., Akilua, A. A., Adenuya, A. A. and Adebayo, M. B. Mineral Contents of Some Commonly Consumed Nigerian Foods.Science Focus.2006,11(1):153-157.
59. Mervat, E.D et al. Effect of fortification by full fat and defatted flaxseed Flour sensory properties of wheat bread and lipid profile laste. Int.J.Curr.Microbiol.App.Sci ,2015, 4(4): 581-598.
60. Chung, M. W., Lei, B., Li-Chan,E. Isolation and structural characterization of the major protein fraction from Nor Man flaxseed (Linumusitatissimum L.). Food Chem.2005, 90, pp.271-279.
61. Land, D.G. and R. Shepherd. Scaling and ranking methods in sensory analysis of foods. 155-185. In. Piggott, J.R. (ed.) Elsevier Appl. Sci., London,1988.
62. Stone H.and Sidel J.L. Sensory evaluations practices, California: Academic Press,1993.
63. AOAC .Seventeenth edition. Official method of analysis. Association of official analytical chemist, Washington D.C. 2000

64. Popa V.M et al. Fatty acids composition and oil characteristics of linseed (Linum Usitatissimum L.) from Romania. Journal of Agroalimentary Processes and Technologies 2012, 18 (2), 136-140.
65. A. Gohari Ardabili, R. Farhoosh, and M. H. Haddad Khodaparast. Chemical Composition and Physicochemical Properties of Pumpkin Seeds (Cucurbita pepo Subsp. pepo Var. Styriaka) Grown in Iran . J. Agr. Sci. Tech. 2011; Vol. 13: 1053-1063

CHAPTER -10

APPENDIX

Department of food technology and Nutrition

Selection of Panel for Sensory evaluation

Sensory evaluation by Hedonic Scale

Name:

Date:

Sample	Appearance	Color	Texture	Taste	Flavor	Overall Acceptability
Control						
BD						
GH						
DE						
AC						

Organolaptic Evaluation:

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

Signature: