ABSTRACT
The work described in the thesis entitled "Studies on the effect of dietary seed proteins and hydrolysates on lipid profile in rats" has been investigated the following aspects.

I) Production of protein isolates and hydrolysates from seed proteins like sesame and mustard and characterization on the basis of various physical properties, amino acid composition, molecular weight distribution etc.

II) Evaluation of the nutritional quality of the protein isolates and hydrolysates in animal model in terms of growth pattern, food efficiency ratio, plasma and tissue lipid profile, corresponding lipid peroxidations, tissue fatty acid composition etc.

Sesame protein isolate (SPI) and respective protein hydrolysates (SPH) are produced from dehulled, defatted sesame seed meal of brown variety. Protein isolates are water washed thoroughly to remove the water soluble polyphenolic components present in sesame meal and SPI has been hydrolysed by papain to produce protein hydrolysate containing short peptides.

Protein content in the SPI and SPH is noted as 91.5% and 93.4% respectively. Crude fibre, ash and phytate content in the SPI have reduced significantly than the sesame meal. PDI and NSI values are also improved remarkably. The polyphenol content in the SPI and SPH is noted as 0.12 % and 0.11% (as gallic acid equivalent) respectively which is in a negligible amount in comparison with the original content of 0.81%.

To get the optimum properties of the sesame protein hydrolysates the hydrolysis reaction has been carried out for 10, 30 and 60 min time and the hydrolysates are designated as SPH$_{10}$, SPH$_{30}$, and SPH$_{60}$.
respectively. After hydrolysis, protein solubility at pH 7.0 increases from 56.9% of SPI to 94.3% for SPH_{60}. Papain hydrolysis significantly improves the emulsifying properties. EAI of SPI increases from 112.5 m^{2}/g to 190.4 m^{2}/g & ESI of SPI from 34.5 to 45.8 min. The protein hydrolysates have been exhibited larger foam capacities (FC) and smaller foam stability (FS) than that of the SPI.

The increase in solubility with the time of hydrolysis is also supported by the degree of hydrolysis. After 60 min of hydrolysis about 25.2% hydrolysis has been observed. Molecular weight distribution of the isolates and hydrolysates are determined by capillary SDS gel electrophoresis. Small peptides of 4-6 KDa are increased in the hydrolysates. To get the maximum benefits from sesame protein hydrolysates the SPH_{60} has been utilized for animal experiment.

Protein content in SPI is 91.5% which is less than that of SPH_{60} (95.04%) and casein (95.7%). Among the amino acids methionine, arginine, serine and glycine content in SPI and SPH_{60} are higher than that of casein, where as lysine, aspartic acid and alanine content is low in SPI and SPH_{60} than that of casein.

Nutritional experiments have been carried out on SPI in comparison with casein with or without cholesterol in the diet. Male albino rats of Charles Foster Strain have been taken for the experiment. All rats have been divided into four groups. Group A (casein, CAS), Group B (sesame protein isolate, SPI) Group C (casein plus cholesterol, CAS-C) and Group D (sesame protein isolate plus cholesterol, SPI-C) have been fed casein, sesame protein isolate, casein with cholesterol and the sesame protein isolate with cholesterol respectively. There is no significant difference in growth rate and FER among the groups during the four weeks experimental period.
The plasma total protein, total cholesterol, HDL-cholesterol, Non-HDL-cholesterol, and triacylglycerol (TAG) level of rats are quantified. Total cholesterol, TAG and non-HDL-cholesterol in the CAS-C group increases significantly than that of normal CAS group. On the other hand, HDL cholesterol decreases in the CAS-C group than that of CAS group. Total cholesterol in the SPI and SPI-C groups decreases at 25% and 29% level than the corresponding control CAS and CAS-C groups respectively. Similarly, TAG content in SPI and SPI-C groups decreases at 27.5% and 48% level respectively than the corresponding control groups. Non-HDL-cholesterol content also decreases in SPI (25%) and SPI-C (38%) groups than the corresponding control CAS and CAS-C groups respectively. There is a significant increase (p<0.05) in the HDL-cholesterol level in SPI (41%) and SPI-C group (38%) than the corresponding control CAS and CAS-C groups respectively. The hypocholesterolemic effect of SPI is prominent both in normal and cholesterol containing groups.

Plasma lipid peroxidation and LOS induced by copper are measured in the four dietary groups. About 49% and 64% lowering of plasma lipid peroxidation, as well as 36% and 56% lowering of LOS in the two experimental groups (SPI and SPI-C) have been observed than the corresponding control (CAS and CAS-C) groups respectively. The lipid profile and the extent of lipid peroxidation in EM ghost of rats are observed. Phospholipid content is more or less similar in all the four groups. Cholesterol content and cholesterol/phospholipid ratio in SPI-C group significantly decreases than the corresponding control CAS-C group. Significant lowering of EM lipid peroxidation (68% and 63% lowering in SPI and SPI-C groups respectively) is also noted in the experimental groups than the corresponding control groups.

Liver weight, total lipid, total cholesterol, phospholipid, triacylglycerol and liver lipid peroxidation of the four groups of rats are
determined. About 24% liver weight has been decreased & total cholesterol, TG levels have slightly decreased in the SPI-C diet than the corresponding CAS-C group diet. There is 52.8% increase in liver phospholipid content of the SPI-C group than the CAS-C group. In vitro liver lipid peroxidation is also significantly decreased (p<0.05) (66% in SPI and 74% in SPI-C groups) in the experimental groups than the corresponding control groups.

The lipid spectrum of heart and brain lipids of the control and experimental groups of rats are also determined. Cholesterol content in the heart lipid of SPI-C group has been decreased significantly (p<0.05) than that of the corresponding control CAS-C group. Phospholipid content in the brain lipid has also been significantly increased (p<0.05) in the SPI-C group than the control group.

Liver lipid fatty acid composition of the different dietary groups has also been determined. In presence of cholesterol in the diet, saturated fatty acid content has been decreased significantly (p<0.05) and corresponding monounsaturated fatty acids have been increased. Among the polyunsaturated fatty acids, n-3 fatty acids have been increased in the SPI and SPI-C group than that of the corresponding control casein groups.

Nutritional experiments have been carried out with sesame protein hydrolysates (SPH₆₀) in comparison with casein with or without addition of cholesterol in the diet. Four groups of rats have been fed with casein (CAS group), sesame protein hydrolysate (SPH group), casein with cholesterol (CAS-C group) and the sesame protein hydrolysate with cholesterol (SPH-C group). There is no significant difference in growth rate and FER between the four groups of rats during the four weeks of experimental period.
The plasma total protein, total cholesterol, HDL-cholesterol, Non-HDL cholesterol, and TAG level of the rats are determined. The total cholesterol, TAG level and non-HDL cholesterol are significantly reduced (p<0.05) in the SPH-C group than the corresponding control CAS-C group. There is a significant increase (p<0.05) in the HDL-cholesterol level in SPH and SPH-C groups than the corresponding control CAS and CAS-C groups respectively. There is significant lowering (p<0.05) of LOS in the two experimental groups (19% in SPH and 26.5% in SPH-C) than the corresponding control CAS and CAS-C groups.

The phospholipid content in EM significantly decreases (p<0.01) in the SPH group than the corresponding CAS group. Cholesterol content in the SPH-C group decreases at 33% level (p<0.05) than the control CAS-C group. EM lipid peroxidation in the SPH group significantly decreases (p<0.01) than the control CAS group.

Liver weight, total lipid, total cholesterol, phospholipid, triacylglycerol and liver lipid peroxidation of the four groups of rats are observed. The weights of liver tissues are significantly increased (p<0.01) in the cholesterol fed groups than the cholesterol free groups. The SPH-C diet significantly decreases (p<0.05) the liver weight than the corresponding CAS-C group. The significant change (p<0.01) in total lipid content is also observed between the SPH group and CAS group. There is a significant increase (p<0.01) in liver phospholipid content of the SPH-C group than the CAS-C group. No significant differences are noted in the in vitro liver lipid peroxidation, total cholesterol and TAG level between the control and experimental groups.

Heart and brain lipid profiles show that there is no significant difference in TAG and phospholipid level for the both control and experimental groups. The cholesterol content in the heart lipid decreases at 52% level (p<0.05) in the SPH-C group than the CAS-C group. The
phospholipid content of the brain lipid increases significantly (p<0.05) both in the SPH and SPH-C groups than the corresponding CAS and CAS-C groups.

We determined the fatty acid composition of the liver lipids of the four dietary groups. Again it has been observed that the saturated fatty acid content in the cholesterol containing groups has been decreased significantly (p<0.05) than the normal groups. Monounsaturated fatty acid content in the cholesterol containing groups (CAS-C and SPH-C) has been increased significantly (p<0.05) than the CAS and SPH groups. Among the polyunsaturated fatty acids, n-3 fatty acid content in the SPH and SPH-C groups have been increased significantly (p<0.05) than the corresponding control groups. DHA content and arachidonic acid (AA, C_{20:4}, n6) content in SPH and SPH-C groups has increased significantly (p<0.05) than the corresponding control groups.

Mustard is another important oil seed crop in India. Protein isolates are prepared from the dehulled, defatted brown mustard seed meal. Mustard protein isolate (MPI) is also washed thoroughly to reduce the polyphenolic components present in it. Respective mustard protein hydrolysates (MPH) are prepared by using the commercial enzyme viscozyme.

Characteristics of the mustard protein isolates and hydrolysates are also reported. The protein content in isolates and hydrolysates are 90% and 94% respectively. Crude fibre content in the hydrolysate is decreased further than that of the respective isolates. Ash content, phytate content, NSI and PDI values are also measured for the mustard meal, mustard protein isolate and mustard protein hydrolysate. The polyphenolic components (as gallic acid equivalent) in the isolates and hydrolysates are also reduced significantly than the mustard meals. The nutritional
effect of the polyphenolic components can be neglected due to very small concentration.

The hydrolysis reaction for the preparation of mustard protein hydrolysate is carried out for three different time intervals by using viscozyme enzyme. The physical properties like solubility, emulsifying properties, and foaming properties of the MPI and different MPH are also observed. The highest % solubility is obtained at 60 min hydrolysis (MPH₆₀) which is 89.3%. Emulsifying properties on the basis of EAI and ESI values are 175.4 m²/g and 40.8 min respectively in case of MPH₆₀. During the preparation of hydrolysates, at 10, 30 and 60 min of hydrolysis, the degree of hydrolysis is noted as 12.8, 14.2 and 16.8 % respectively. By considering the properties of the mustard protein hydrolysates at three different time intervals the MPH₆₀ has been utilized for animal experiment.

The amino acid compositions of the MPI and MPH₆₀ along with ash, fibre and protein content in comparison with casein is determined. Protein content in casein, MPI and MPH₆₀ is noted as 95.7%, 90.0 % and 94.0% respectively. Among the amino acids cystine, proline, histidine and arginine content in MPI and MPH₆₀ are higher than that of casein. On the other hand valine and aspartic acid content in MPI and respective MPH₆₀ is lower than that of casein.

Four groups of rats are fed with casein (CAS group), mustard protein isolate (MPI), casein with cholesterol (CAS-C) and mustard protein isolate with cholesterol (MPI-C). There is no significant difference in growth rate and FER among the groups during the four weeks of the experimental period.

Plasma total protein and lipid profile of rats raised on casein (CAS group), mustard protein isolate (MPI), casein containing 2% cholesterol (CAS–C group), and MPI containing 2% cholesterol (MPI–C group) are also observed. Diet containing 2 % cholesterol again significantly increases
(p<0.05) plasma total cholesterol, TAG, and non-HDL-cholesterol level both in control and experimental groups than the corresponding cholesterol free groups. About 13.5% and 35% of the total cholesterol has been decreased in the experimental groups than the corresponding control groups.

TAG level in plasma is significantly reduced (p<0.05) in the MPI and MPI-C group than the corresponding control CAS and CAS-C groups. There is 37.5% and 44.85% increase in the HDL-cholesterol level has been observed in MPI and MPI-C groups than the corresponding control CAS and CAS-C groups respectively. Non-HDL-cholesterol in the MPI-C group has also been decreased significantly (p<0.05) than the control CAS-C group.

Significant lowering of LOS (p<0.05) in the MPI and MPI-C groups (43.75% and 67.97% respectively) has been observed than the corresponding control CAS and CAS-C groups. No significant differences are noted in plasma lipid peroxidation level between the control and experimental groups.

In the isolated EM, the phospholipid, cholesterol content and cholesterol/phospholipid ratio are more or less similar for the both control and experimental groups (with or without cholesterol). EM lipid peroxidation in the MPI and MPI-C group is significantly decreased (p<0.05) than the control CAS and CAS-C groups.

Liver weight, total lipid, total cholesterol, phospholipid, TAG and liver lipid peroxidation of the four groups of rats are also reported. The weights of liver tissue are significantly increased (p<0.05) in the cholesterol fed groups than the corresponding cholesterol free groups. The MPI–C diet significantly decreases (p<0.05) the liver weight than the corresponding CAS–C group. Significant decrease (p<0.02) in total cholesterol content is also observed in MPI group than the CAS group. Liver phospholipid
content of the MPI group has been increased at 20% level (significant, p<0.02) than the CAS group. TG content in the liver lipid is also decreased significantly (29.5% and 18.2% respectively) in the MPI and MPI-C groups than the control CAS and CAS-C groups respectively. Significant decrease in liver lipid peroxidation (p<0.05) in the MPI-C group than the control CAS-C group has been observed. HMG-CoA and mevalonate ratio of different dietary groups is also studied.

After 4 weeks of animal experiment the rat livers are examined histologically. The cholesterol deposition in the rat liver is distinctly visible in CAS group and CAS-C group, whereas in MPI and MPI-C groups very small amount of cholesterol deposition has been observed.

The cholesterol content in the heart lipid decreases at 22.3% and 30.35% level in a significant manner in the MPI (p<0.001) and MPI-C group (p<0.05) than the corresponding CAS and CAS-C groups. TAG content in the heart lipid also decreases at 36.5% and 25.63% in MPI and MPI-C groups than the corresponding control groups respectively.

The brain weight, brain lipid content and lipid composition of the different dietary groups are estimated. The phospholipid content of the brain lipid increases significantly both in the MPI (32.11%, p<0.02) and MPI-C (15.14%, p<0.05) groups than the corresponding CAS and CAS-C groups.

Saturated fatty acid content in the liver lipid of cholesterol containing groups decreases and the corresponding monounsaturated fatty acid content increases in a significant manner (p<0.05). Among the polyunsaturated fatty acids, n-3 fatty acid content in the MPI and MPI-C group increases significantly than the corresponding control CAS and CAS-C groups and n-6 fatty acid content in the cholesterol supplement group increases significantly (p<0.05) than the corresponding cholesterol free groups.
Nutritional experiments are also carried out with mustard protein hydrolysate in comparison with casein in the hypercholesterolemic diet. Only hypercholesterolemic diets (2% cholesterol) are considered to evaluate the nutritional aspects of mustard protein hydrolysate. The control group is fed with casein containing 2% cholesterol (CAS-C) and the experimental group is fed with mustard protein hydrolysate (MPH) containing 2% cholesterol (MPH-C). There is no significant difference on growth rate and food efficiency ration between the control and experimental groups of rats.

The plasma total protein, total cholesterol, HDL-cholesterol, non-HDL cholesterol, and TAG level of rats raised on casein containing 2% cholesterol (CAS-C group), and MPH containing 2% cholesterol (MPH-C group) are determined. The total cholesterol, TAG level and non-HDL-cholesterol levels are significantly reduced (p<0.001) in the MPH-C group than the corresponding control CAS-C group.

The plasma lipid peroxidation and LOS induced by copper in the two dietary groups show that no significant differences are noted in plasma lipid peroxidation level between the control and experimental groups. Significant lowering of LOS (44.05%, p<0.001) in the experimental group (MPH-C) has been observed than the corresponding control (CAS-C) group.

The lipid profile and the extent of lipid peroxidation in EM ghost of rats show that the cholesterol content in the MPH-C group decreases significantly (p<0.001) at 44.82% level than the control CAS-C group. Significant decrease (p<0.001) has also been observed in EM lipid peroxidation in the MPH-C group (at 43.49% level) than the control CAS-C group.

Liver weight, total lipid, total cholesterol, phospholipid, triacylglycerol, liver lipid peroxidation and HMG-CoA & Mevalonate ratio
of the two groups of rats are noted. The weight and lipid content of liver tissues are significantly decreased ($p<0.01$) in the experimental cholesterol fed (MPH-C) group than the cholesterol fed control group (CAS-C). There is significant decrease in liver cholesterol, triacylglycerol of the MPH-C group than the CAS-C group.

The significant decrease in total lipid content ($p<0.02$), total cholesterol ($p<0.05$) and triacylglycerol level ($p<0.001$) has been observed in the heart lipid in the MPH-C group than the CAS-C group. The brain weight, total lipid, total cholesterol and phospholipid content in the experimental MPH-C group increase to some extent but it is not significant.

The fatty acid composition of the liver lipids of the two dietary groups show that there is no significant difference in fatty acid composition between the two groups, only a trend of increase in arachidonic acid ($C_{20:4}$, n-6) and DHA ($C_{20:6}$, n-3) have been observed.

The overall investigations have generated a number of nutritional information on two important vegetable protein sources like sesame and mustard meal which can be utilized as therapeutic protein in various food formulations.