CHAPTER VIII

SUMMARY AND CONCLUSIONS
Green biomass in general and photosynthetically active green leaves in particular, constitute world's largest supply of usable protein. However, as proteins in leaves are associated with indigestible fibrous or cell wall material, these as unacceptable as human food. Out of the total proteins synthesised in leaf, only 20-30 % of them can be recovered by harvesting grains, tubers, roots and other food products of plant origin. The efficiency of protein recovery further decreases when food products of animal origin (e.g. milk, meat) are considered. In this context, the suggestion made by Prof. N.W. Pirie that it might be advantageous to extract protein directly from leaves has recently gaining acceptance for increasing overall efficiency of protein utilization. For this purpose, he suggested the technique of green crop fractionation (GCF), the theme on which present investigations were undertaken.

During GCF, fresh green leaves are macerated to rupture the cells and subsequently pressed. The juice released due to the pressing is then heated or acidified, as a result of which protein in leaf juice denature, precipitate and
coagulate to form a curd referred to as leaf protein concentrate (LPC). The proteins in LPC are associated with other plant nutrients, for which it is also known as protein mineral vitamin concentrate or leaf nutrient concentrate (LNC). As proteins in leaf are mostly enzymes and metabolically active, these are nutritionally superior. The LPC prepared from leaves is suitable as a source of protein and vitamin A in human nutrition and its use in the diet has been widely recommended. It is believed that extraction of proteins from green leaves may supply abundant and cheap protein source for increasing population, and particularly to overcome protein calorie malnutrition (PCM).

Studies on various aspects of green crop fractionation (GCF) and leaf protein (LP) were initiated in this Department in 1967. The results obtained during last 30 years gave a strong evidence that, with GCF overall efficiency of agricultural land utilization may increase. Several plant species were found suitable for LP extraction. It was also pointed out that the residue remaining after extraction of juice from leaves is a suitable feed grade product. It has also been demonstrated that the process of fractionation can be undertaken in rural areas without disturbing prevalent agricultural and dairy practices. The investigations undertaken during present study and reported in the dissertation forms a part of the research programme on LP being in progress at this Department.

Choice of suitable species for protein extraction forms an important part of the investigations on leaf protein. Dark green leafy vegetables (DGLV) are potential sources of proteins, vitamins and minerals. In view of their popularity
it was thought worthwhile to cultivate them for harvesting their foliages to prepare LPC and undertake studies on measurement of yields. Ten vegetable crops were cultivated in the University Botanical Garden during October 1997 and August 1998, harvested for green foliage at pre-flowering stage and yields were recorded. It was interesting to note that the yield of by-product leaves of radish was highest among leafy vegetables, inspite of the production of edible roots. The three leguminous vegetable crops mungbean, mothbean and cowpea yielded maximum crude protein per hectare, which was followed by the yield of crude protein from by-product leaves of radish. Fenugreek yielded maximum LPC per unit weight of the green foliage with 58.3% crude protein content. Among all vegetable crops, the performance of Rumex was poor. All crops, except Rumex, yielded appreciable amounts of LPC with maximum recovery of proteins in fenugreek and spinach. Based on the results obtained with the vegetable crops, which are presented in chapter III, it is concluded that by-product leaves of radish and the leaves of leguminous vegetable crops could be employed for leaf protein production.

Apart from choice of the vegetation for leaf protein (LP) extraction, machinery suitable for fractionation (i.e. maceration and pressing) also play an important role in producing feed and food grade products from green leaves. Preliminary investigations in this Department were undertaken using domestic appliances. Subsequently the Department received a pulper and laboratory scale press as a gift in 1969 from International Biological Programme (IBP). During the attempts to produce LPC on farm, a screw press was fabricated in
the University Central Workshop for simultaneous maceration and pressing of the leaves. The performance of sugarcane crusher was also evaluated during earlier studies. Apart from commercial production of leaf protein, the author thought worthwhile the need of producing LPC on small scale domestic level using locally fabricated / manufactured machinery. A small hand operated single screw press was fabricated for this purpose with the help of Graduate Students of M.I.T. College of Engineering and was modified in the University Central Workshop. To evaluate performance of lucerne (Medicago sativa L.) foliage with newly fabricated screw press, as well as locally popular sugarcane crusher, the crop was fractionated for 10-12 times and the distribution of dry matter (DM) and nitrogen (N) in various fractionation products was studied. When the foliage of lucerne was fractionated on sugarcane crusher during February and June, 1998, satisfactory results were obtained. The crop resulted in feed grade product with 32 % dry matter (DM) and 16% crude protein in the DM; along with a food grade LPC with 40% DM and 55% crude protein. When the crop was fractionated on hand operated screw press for 12 times during January 1 and May13, 1998, the fractionation was equally successful producing feed grade pressed crop residue and food grade leaf protein concentrate of equivalent quality. The results obtained which are presented in Chapter IV indicated that the two machines i.e. sugarcane crusher and hand screw press were suitable for leaf protein extraction.

The performance of sugarcane crusher and hand operated screw press for leaf protein extraction from lucerne was compared with the machinery used
by earlier workers. On the basis of proportions of dry matter (DM) and nitrogen (N) distributed in fractionation products, it was concluded that the performance of these two machines was comparable to IBP pulper-press combination.

After obtaining satisfactory results with the two machines (single screw press and hand screw press) for fractionation of lucerne, it was thought reasonable to fractionate foliages of other species on these machines to study their overall performance for fractionation purpose. For this purpose 10 crops were selected and fresh foliages obtained from them were fractionated simultaneously on IBP pulper-press, sugarcane crusher and hand screw press. The results obtained are presented in chapter V, which indicated suitability of these two new machines for fractionation. While comparing the results obtained using these machines with those obtained on IBP pulper-press, it was pointed out that the hand screw press is as efficient as IBP equipment for fractionation of green foliage from fenugreek, mungbean, mothbean and cowpea; while sugarcane crusher for the fractionation of Dolichos, mungbean, mothbean and cowpea. While fractionating the foliages, it was experienced that state and texture of the crop largely influence its fractionation with these machines. It was concluded that these two machines could be employed for fractionation with minor modifications and adjustments, depending on the crop to be employed for leaf protein extraction.

The fractionation of any green foliage and methods employed for it largely depends on:
i) Whether maximum proteins are to be extracted to recover optimum leaf protein concentrate, considering it as a main product and pressed crop residue for animal nutrition as feed grade by-product;

ii) Whether only the excess proteins are to be extracted along with the moisture, and LPC be considered as by-product and feed grade pressed crop residue as a main product; or

iii) Whether both the products i.e. food grade LPC and feed grade pressed crop residue are to be considered as co-products to fulfil the requirements of human beings as well as animals.

In view of these facts it can be concluded that appropriate machine and raw material in the form of green leafy biomass should be selected keeping in view the intention of fractionating them.

Untraditional sources of protein, other than leaf protein, have also been recommended to overcome protein deficiency in human diet. Attempts have been made during present investigation to compare nutritive value of LPC with yeast (a single cell protein source) and algal biomass. The results obtained suggested nutritional superiority of LPC over other sources. In addition, the experiments conducted with the preservation of LPC indicated that the wet LPC can be preserved with acetic acid and salt. The use of domestic additives for preservation gave discouraging results.

After isolating LPC from heated juice, the deproteinized juice (DPJ) is released as a by-product of GCF system. The DPJ is a liquid by-product
containing soluble plant nutrients, and its disposal as an affluent may cause bio-pollution. To avoid this, several uses of DPJ have been advocated which include its recycling back to the pressed crop residue for animal feeding or to the soil as a source of fertiliser. It is a best medium for growing microorganisms and can be employed in microbial biotechnology to produce organic acids, enzymes, antibiotics, toxins, single cell protein etc. Earlier investigations in this laboratory, however, indicated that the DPJ from lucerne cause chromosomal aberrations in the root tips of onion and prevent mitotic cell division. At higher concentration, it inhibits seed germination, although its use as a constituent in the medium for tissue culture has been recommended. During present investigation the effect of DPJ obtained from foliages of seven crops was tested on germination of ten types of seeds. The results obtained supported earlier conclusion that DPJ inhibits seed germination. It was, however, pointed out that the effect of DPJ is species specific. From these primary investigations presented in Chapter VII, it can be concluded that the antinutrients, salts and secondary metabolites in DPJ are probably responsible for inhibiting germination of seeds.

The overall results presented in the thesis indicated wide scope for undertaking fractionation of leaf materials, particularly on small scale domestic level, to provide protein rich diet which is also rich in vitamin A. It is felt that the investigations reported will help in critically understanding the concept of green crop fractionation.