SUMMARY AND CONCLUSIONS

Food-based strategies of vitamin A deficiency, especially the inclusion of easily accessible, inexpensive β-carotene rich plant foods, namely the GLVs in the everyday diet, are currently being propagated and promoted in developing countries to alleviate the micronutrient deficiencies, particularly vitamin A deficiency, among the vulnerable groups. However, several gaps of knowledge exist which need to be addressed for proper implementation of this strategy. In light of this, the objectives of this study were:

1. To develop an inventory of the key β-carotene rich foods consumed in the Western Indian State of Gujarat.

2. To analyze β-carotene content of the selected seventeen GLVs by high performance liquid chromatography.

3. To analyze selected nutrients and antinutrient in these GLVs.

4. To test the bioavailability of β-carotene from one leafy vegetable rich in β-carotene (fresh and dehydrated drumstick leaves) and compare it with that of synthetic vitamin A in a vitamin A deficient rat model.

5. To formulate recipes using an underutilized leafy vegetable (fresh radish leaves) involving three different cooking methods and study the retention of β-carotene in them.

The thesis is divided into eight chapters. Chapter I is an introduction to the study. Chapters II – VI outline the results of the five sub-studies described above. They all follow a uniform format of a short review followed by methods and materials and results and discussion. The major findings section-wise are summarized below.
SECTION I

- An inventory of β-carotene rich foods was developed for the Western Indian State of Gujarat using qualitative research methodologies such as market surveys, key informant interviews and direct observations.

A wide variety of carotene rich foods 41 in all, were available in this Western region of India on a year-around basis although not all were sold through the market. The tribal population used several uncommon leafy vegetables such as kuba (*Leucas aspera*), dodi (*Pergularia daemia*) and poi leaves (*Basella rubra*), that must make some contribution to the vitamin A intake. The main food sources in the rural areas included cultivated fields, markets, home gardens, gatherings from the nearby bushes or fences and plucking GLVs grown as secondary crops along with the primary crops. In the urban areas, the markets were the main sources of vegetables. Of the 41 carotene rich foods identified, 25 were available during the winter months (November - February) in the urban market. However, the accessibility of GLVs in summer (March - June) and the monsoon (July - October) season was relatively scarce. It was noted that the GLVs were prepared using several methods such as boiling, sautéing, steaming, roasting, baking and deep-frying. Several GLVs such as radish leaves, amaranth, spinach, fenugreek, cabbage and coriander, were prepared in combination with cereal-pulse mixes.

Since several health-promoting properties were ascribed to these GLVs by the rural and tribal people, these positive beliefs could be used for the promotion of these GLVs to combat VAD. Many of the GLVs consumed in the rural and tribal areas were not listed in the food composition tables of India. Data for their β-carotene content and other chemical constituents were also not available which prompted the studies in section II and III.
The β-carotene content of 17 indigenous GLVs, selected on the basis of availability and frequency of consumption, was estimated using a non-aqueous reverse-phase HPLC method. A ternary mixture of acetonitrile, dichloromethane and methanol (70:30:10) was used to separate β-carotene isocratically on a octadecysilane (C18) column at 450 nm.

The HPLC separation and quantification of β-carotene showed that 8 of the 17 GLVs had a β-carotene content of more than 5000 μg/100g FW. Drumstick leaves (Moringa oleifera), kanjaro (Digera arvensis) and fenugreek leaves (Trigonella foenum graecum) were the best sources of provitamin A activity with a β-carotene content of 19210 μg/100g; 14390 μg/100g and 10226 μg/100g FW respectively. The lowest amount of β-carotene was found in luni (1195 μg/100g FW). β-carotene expressed as percent total carotene ranged from 8.2% to 93.6% in these leaves.

The results of the β-carotene analysis revealed that all the GLVs except luni had a β-carotene content in excess of 1600 μg/100g FW, which is equivalent to the recommended dietary allowances for Indian children (ICMR, 1989). Even after accounting for about 50% losses in processing of the GLVs, 11 of the 17 GLVs could provide 1600 μg/100g, which included chana leaves, chil, dodi, drumstick leaves, fenugreek leaves, jharakhala, kanjaro, khatedo, poi, radish leaves and shepu.

The nutritional significance of these findings is clear; that the GLVs, especially the indigenous, uncommon ones could be important sources of vitamin A for major communities of India. Among these GLVs, drumstick leaves, though not commonly consumed, offers the best potential for use as a carotene supplement, in view of its very high β-carotene content.
SECTION III

- The analysis for moisture, ash, total iron, calcium, phosphorous, ascorbic acid and oxalic acid was done using standard procedures, in the seventeen selected GLVs.

The chemical analysis of the seventeen GLVs revealed that the iron content of the leaves varied from 0.2-0.2.2 mg/g dry weight. Calcium and phosphorous values were between 2.2-42 mg and 2-12 mg/g dry weight respectively. Forty-five percent of the leaves had a desirable Ca: P ratio of 2:1. The ascorbic acid content of these leaves ranged from 2-11.4 mg/g dry weight. Only some of the leaves had a desirable Ca: Oxalic acid ratio (2:1). Due to the high oxalic acid content many leaves were ranked low in their nutritional value. This study signifies the importance of the analysis of the GLVs for the antinutrients especially oxalic acid before ranking them for their nutritional significance, thus, increasing their usefulness in evaluating the food consumed by the people in this region.

This study also added a database for eleven GLVs whose values were not available in the food composition tables of India (NIN, 1993).

The chemical composition of the GLVs namely the ascorbic acid, iron, calcium, phosphorous and the oxalic acid results of the current study has thus enabled us to rank them as per their quality for human consumption. Based on the criteria of a value of iron >0.7 mg/g DW, the ascorbic content 0.6 mg/g DW, a desirable Ca: P and Ca: oxalic acid ratio of 2:1 - five of the seventeen green leafy vegetables ranked as very good.
These were:

1. Drumstick (*Moringa oleifera*)
2. Fenugreek (*Trigonella foenum graecum*)
3. Onion stalks (*Allium cepa*)
4. Radish leaves (*Raphanus sativus*) and
5. Kuba (*Leucas aspera*).

The β-carotene content of these leaves was 19210 μg/100g, 10226 μg/100g, 2970 μg/100g, 6540 μg/100g and 3066 μg/100g fresh weight respectively.

Some of these are grossly underutilized, for eg., drumstick leaves. They are not only rich in certain essential nutrients, but are also practical and economical for menu planning consistent with local eating habits. Such GLVs should be advocated for greater use, and some would be valuable in formulating infant foods and or protein supplements.

The regional variations that became apparent when the present study results were compared with other studies in the literature, emphasized the need to generate a database of the available foods of each region before compiling and editing the food composition tables of India, (a country, with a variety of soil, climate and topography). Preservation techniques and bioavailability trials also need to be conducted for these GLVs which offer themselves as excellent candidates for combating micronutrient malnutrition.

SECTION IV

- Bioavailability trials using fresh and dehydrated drumstick leaves were conducted on vitamin A deficient rats. Male albino *Charles foster* strain of rats were fed on a synthetic vitamin A deficient diet for 4 weeks and thereafter repleted with fresh or dehydrated drumstick leaves or synthetic vitamin A (vitamin A acetate) for the following 4 weeks. These were compared with a
control group of rats fed on a basal diet adequate in vitamin A for 8 weeks. The diets were iso-caloric and iso-nitrogenous. The parameters tested were: weight change, food intake, clinical signs and symptoms, organ weights, serum and liver retinol levels.

The results of the bioavailability trials revealed that, marked clinical signs of vitamin A deficiency, reduced food intake, weight loss, decline in serum vitamin A (19.1 μg/dl) and hepatic vitamin A (2.0 μg/g liver) was observed in rats after 4 week of vitamin A deficient diet. Compared to these, the control rats gained weight and had adequate serum (38.4 μg/dl) and hepatic vitamin A (7.1 μg/g liver). Repletion with either synthetic vitamin A or drumstick leaves in fresh as well as dehydrated form, brought about significant improvement in the clinical signs and symptoms, body weight and food intake of the vitamin A repleted rats. The serum vitamin A levels of the fresh (25.8 μg/dl) and dehydrated drumstick leaf groups (28.2 μg/dl) were lower than that of the synthetic vitamin A group (34.7 μg/dl). However, compared to the values at the end of 4 weeks of depletion (19.1 μg/dl), these were significantly higher. The liver retinol values showed a significant improvement in all the three repleted groups as compared to the vitamin A depleted group (2.0 vs 5.5; 4.8 and 6.0 μg/g liver for vitamin A deficient, synthetic, fresh drumstick and dehydrated drumstick leaves group respectively).

The results imply that foods high in carotenoids are as effective in overcoming vitamin A deficiency as pure synthetic vitamin A. These foods also have an advantage over purified supplements since they also provide many other biologically active constituents that are not present in purified micronutrient supplements. These constituents also may be important for contributing to optimal health. Food sources of β-carotene also have advantages over the synthetic vitamin A in terms of their low toxicity, easy availability in the developing countries and low cost.
Since the food supplements of β-carotene had a therapeutically similar effect to vitamin A acetate in vitamin A deficient rats, the study has important public health implications in the struggle against vitamin A deficiency. Beta-carotene from the fresh and dehydrated drumstick leaves could be given to children, pregnant and lactating women to increase their body stores since β-carotene is safer than synthetic vitamin A. Fresh drumstick leaves are widely found in most of the Indian subcontinent, and the preservation of these could be easily accomplished by shade dehydration at the household level with the use of simple technology (blanching and sulphiting). Due to the high bioavailability exhibited by these GLVs, in the present study, it can be concluded that nutrition education leading to an increased consumption of these GLVs would go a long way in eliminating vitamin A deficiency. Additional research to understand details of efficacy under field circumstances more quantitatively are warranted but are not necessary before deciding on and implementing carotene-containing food-based interventions.

SECTION V

- Three cereal/pulse-based recipes were standardized so as to incorporate maximum amount of fresh radish leaves and had the highest organoleptic scores. These were: dhebra (shallow fried) with 75 g of fresh radish leaves/100 g of the mix; muthia (steamed) with 75 g of fresh radish leaves/100 g of the mix and handwa (baked) with 40 g of fresh radish leaves/100 g of the mix. The retention of β-carotene in these three products was also estimated.

All the three products dhebra, muthia and handwa, which were developed after incorporation of fresh radish leaves, were acceptable to the panel of judges with the composite scores being 4.8, 4.5 and 4.0 respectively. The same recipes when tested using the 9-point hedonic scale revealed similar results with scores ranging from 8.5 to 7.3; the highest ranking product was dhebra with a score of 8.5, followed by muthia with a score of 8.3 and the lowest ranking was handwa with a score of 7.3.

161
Thus judging from the results of the amount of leaves that could be incorporated, acceptability characteristics dhebra ranked first followed by muthia and handwa. The percent retention of β-carotene in these products revealed that the shallow-fried dhebra retained the maximum amount of β-carotene (82%) as compared to steamed and sauteed muthia (68%) and baked handwa (36%).

The nutritive value of these recipes per serving were equivalent to that recommended by the ICDS for the supplementary feeding program for children, namely each serving provided ≥ 300 Kcal and 6 g protein. In addition they provided 80% or more of the RDA for β-carotene.

Promotion of consumption of these β-carotene rich products for preschool children through supplementary feeding programs, appears to be the right step to improve vitamin A status of the children. These results also indicate that education designed to practice effective cooking methods will contribute towards increased intakes of β-carotene.
CONCLUSIONS

1. The Western Indian State of Gujarat has a wide variety of carotene rich foods but many of them are under-explored as far as human consumption and vitamin A deficiency are concerned.

2. Several of the GLVs identified had not been analyzed for either carotene or other nutritional constituents and such an analysis performed on 17 GLVs showed that there were many GLVs grown in tribal and rural areas which are not available in the markets and which could serve as valuable source of vitamin A during the season of availability.

3. Chemical analysis indicated that these GLVs were rich in minerals such as calcium, iron, ascorbic acid and will have been adequately treated to remove their oxalic acid.

4. Drumstick leaves, which contain a very high amount of β-carotene are highly bioavailable in fresh as well as in dehydrated form, as shown by the rat model. The drumstick trees, which are currently promoted under trees for life program and other social forestry schemes, can be utilized very profitable in supplementary feeding programs.

5. The products developed using one of the GLVs has relatively high retention of β-carotene, thus confirming that provitamin A foods hold the promise for the virtual elimination of vitamin A deficiency, a goal enunciated in the national plan of action for Nutrition in India.

6. Most importantly, the bioavailability trials have highlighted that plant carotenoids are not inferior to synthetic vitamin A in combatting vitamin A deficiency.