CHAPTER I

INTRODUCTION

THE PROBLEM OF VITAMIN A DEFICIENCY IN INDIA

Vitamin A deficiency (VAD) has long been identified as a serious and preventable nutritional disease, which even in its relatively early stages results in an impairment in linear growth, cartilage and bone development and epithelial cell differentiation and functions in animals (Roberts and Sporn, 1984) and in reduced vision in dim light in humans (Underwood, 1984). Vitamin A deficiency affects the epithelial structures in various organs, the eye being the most obvious and striking (FAO/WHO, 1992).

As the affliction continues, the eye's conjunctiva and cornea become dry, lesions then appear on the cornea which in the severest cases is succeeded by permanent blindness (Sommer, 1982).

Vitamin A cannot be synthesized de novo and therefore it must be provided from the diet (Simpson, 1983). The major cause of VAD in general is the inadequate consumption of retinol/carotene rich foods (Narasinga Rao, 1991). Increased vitamin A requirements in certain physiological or pathological conditions, inadequate absorption, or loss of intestinal contents in diarrhea are also contributory factors to vitamin A deficiency (Underwood, 1984). Low dietary intake of vitamin A and its precursors results in depletion of liver reserves of vitamin A over a period of time (Sommer, 1982). India is a country with rich biodiversity and is bestowed with a wide array of plant foods (Pandey, 1995). Thus, the prevalence of xerophthalmia in India and other south-east Asian countries, has the unusual characteristic of usually being the disease of “poverty in the midst of plenty” (Wasantwisut, 1995); where it is a serious problem there is no lack of provitamin A, in the form of dark-green leafy vegetables (Narasinga Rao, 1991). The proper use of these cheap and
readily available sources must be the cornerstone of the public health approach to combat VAD (Vijayaraghavan, 1992).

INTERVENTIONS TO COMBAT VITAMIN A DEFICIENCY

The main intervention strategies to combat vitamin A deficiency include:

1. Dietary diversification: increased consumption of vitamin A rich foods, change in the current dietary habits through various means, which includes promotion of home gardening/cultivation of beta carotene rich foods and nutrition education;
2. Food fortification (with vehicles such as salt, sugar and monosodium glutamate etc.);
3. Direct supplementation of vulnerable populations or groups with synthetic vitamin A and
4. Public health strategies (such as improved sanitation and hygiene).

Of the above strategies, dietary improvement aims to increase dietary availability, regular access to and consumption of low-cost vitamin A rich foods or changing the intra-family distribution of such foods among the at-risk populations (Obaidullah Khan, 1995). Such efforts involve improving the overall diet through changes in dietary behaviour of the targeted population. Food-based strategies have the long term potential to solve the problem of VAD as poor diet is the root cause of this deficiency (Smitasiri et al., 1992). Poor diets also contribute to VAD especially when they are low in fat, protein and zinc, or essential nutrients necessary for absorption and utilization of vitamin A (Lala and Reddy, 1970). Surveys in India have shown a low intake of retinol 60-100 μg/d as against the RDA of 350 μg/d in preschool children (Reddy, 1991). Plant foods, rich in carotenoids or provitamin A, could play an important role in combating VAD in Indian population which is largely vegetarian (Gopalan, 1996).
Carotenoids by themselves are also being credited with other important health promoting effects, independent of their vitamin A activity, such as prevention of cancer, immuno-enhancement, and prevention of cardiovascular disease, cataracts, and muscular degeneration (Mathew-Roth, 1985; Krinsky, 1988). Thus, a sustainable diet/food-based approach, which would improve not only vitamin A status but also the overall nutritional status is the most rational means to combat VAD in India (Gopalan, 1996).

AN INVENTORY OF CAROTENE RICH FOODS: A NECESSARY CONCOMITANT TO DIETARY DIVERSIFICATION

India is bestowed with a wide range of carotene rich foods. However, the per capita availability of vegetables and fruits is only 50% of that recommended by the Indian Council of Medical Research (ICMR, 1989). The recommended intake of β-carotene from vegetables per caput per day is 2290 μg whereas the average intake from fruits and vegetables is only 1160 μg showing a shortfall of 1130 μg per day (Narasinga Rao, 1991). The above estimate is based on market supplies which do not consider several edible, seasonal semi-domesticated and the under-exploited plant foods (also known as the poor man's vegetables). Several of these plant foods are yet to be identified and documented for their nutritional contribution.

Thus one of the prime research needs is to develop a region wise inventory of the carotene rich foods that are available and consumed in different seasons in India. It is also important to identify what vitamin A rich foods could serve as important sources of vitamin A for children (Seshadri, 1996).

The first section of the present study addressed this need in the Western Indian State of Gujarat. Two districts from the state of Gujarat were selected and a set of qualitative tools such as market surveys, key informant interviews and household interviews were used to collect data for the development of the inventory.
NEED TO GENERATE A DATABASE ON BETA CAROTENE CONTENT BY HPLC

Most vegetables contain a number of different carotenoids. Among these β-carotene has the highest vitamin A activity and is most widespread, although its concentration varies considerably (Bauernfeind, 1972). Alpha-carotene, the 9 and 15 cis isomers of β-carotene, β-cryptoxanthin and γ-carotene are minor components. At present the number of naturally occurring carotenoids exceeds 600 (Straub, 1988) whereas the number of vitamin A active compounds is only a fraction of this (Bauernfeind, 1972). It is generally recognized that, in terms of biological activity, 1 µg of retinol is equivalent to 6 µg of β-carotene or 12 µg of mixed carotenoids (WHO, 1967; Food and Nutrition Board, 1980).

In view of the important role played by the vitamin A active carotenoids in human nutrition, an accurate assessment of the provitamin A content of foods is becoming increasingly important (Khachik et al, 1991). Much of the ‘carotene data’ in the Food Composition Tables have been obtained by measuring total absorption at a specified wavelength and quantified against β-carotene, or by open column chromatography (AOAC method, 1984). Several studies have pointed out the need to update these data (Lee et al, 1992, Speek et al, 1986; Udipi et al, 1982; Reddy et al, 1995) because column chromatography often fails to resolve the most potent vitamin A precursor, all trans-β-carotene from its less active geometrical isomer α-carotene (Steward, 1977) and lacks reproducibility and accurate quantitation. Thus it could overestimate the β-carotene value of the foods. The advent of high performance liquid chromatography (HPLC) which makes available rapid, reproducible, quantitative and accurate analysis has opened up new possibilities for the study of carotenoids (Sweeny and Marsh, 1971; Bushway and Wilson, 1982).

Besides the need to obtain data by HPLC, there are several unconventional carotene rich foods identified in India, whose β-carotene
content is not reported (Reddy, et al, 1995; Seshadri, 1996). The second section of the present study addressed this objective of determining the β-carotene content, by HPLC, of some of the key carotene rich foods consumed in Gujarat as identified in section I.

NEED TO GENERATE DATA ON CONTENT OF OTHER NUTRIENTS AND THE ANTINUTRITIONAL FACTORS OF THE CAROTENE RICH FOODS

The usefulness of plant foods as sources of micronutrients can be clearly delineated. Poor people obtain most of their nutrients from plant foods, which are cheaper and more accessible than animal foods (Gopalan, 1996). Plant foods especially GLVs, besides being rich sources of beta carotene, also contribute significant amount of other minerals such as iron, calcium, phosphorous, zinc and provide substantial quantities of vitamins such as ascorbic acid and folic acid (Faboya, 1983). The nutritional contribution of the GLVs is dependent on both the nutrient as well as the antinutrient profile of these. Many of the GLVs are found to be high in antinutritional factors such as oxalic acid (Zarembski, 1962; Singh et al, 1970; Wilson et al, 1982) which may seriously interfere with the absorption and bioavailability of minerals particularly calcium, magnesium (Hogkinson, 1977; Pingle, 1978; Narasinga Rao, 1982; Heaney and Weaver, 1989), and iron (Chawla et al, 1988, Siegenburg et al, 1991). Since data on these constituents for several of the provitamin A foods especially, the indigenous GLVs, is unavailable and is often incomplete for the common leafy vegetables, a need to analyze the nutrient and antinutrient profile of these exists. The third section of the study thus focussed on the chemical analysis of several of the GLVs identified in section I for selected nutrients and antinutrients.

IS BETA CAROTENE READILY AVAILABLE FROM GLVs?

There is sufficient evidence to conclude that food-based approaches using provitamin A sources, when adequately implemented, are effective in
the control of VAD, and contribute to alleviating the other usual accompanying nutritional deficits, e.g., of other micronutrients such as iron and protein (Devadas et al, 1978; Jayarajan et al, 1980; Carlier et al, 1991; Carlier et al, 1992). Evidence comes from vegetarian populations that do not show vitamin A deficiency, from small scale community-based studies that have fed controlled amounts of carotene sources and demonstrated elimination of subclinical deficiency (Oey Khoen Lian et al, 1967; Venkataswamy et al, 1976; Sharma et al, 1993) and from clinic-based metabolic studies on absorption from different carotenoid sources (Lala and Reddy, 1970; Hussein and El-Tohamy, 1990). In certain cases where protein is limiting in the diet, β-carotene consumption may be preferable to large doses of retinyl palmitate, since carotenoids generate retinoids which are not protein-dependent for mobilization (Carlier et al, 1992). However, certain studies have reported that provitamin A from food sources are reported to be inefficiently absorbed and metabolized, especially when compared to synthetic β carotene in oil or in antioxidant stabilized commercial forms (Bauernfeind, 1981; Bulux, 1993; de Pee et al, 1995).

The bioavailability from pure β-carotene is not necessarily representative of the β-carotene in foods as the situation is entirely different when the provitamin is assayed in a complex matrix such as food samples containing active, moderately active and inactive carotenoids (West, 1997). The bioavailability of β-carotene from plant foods, especially GLVs is also affected by many other factors such as the species of carotenoids, molecular linkage, matrix in which it is incorporated and absorption modifiers (de Pee et al, 1995; West, 1997; Reddy, 1995). Therefore, while the analysis of green leafy vegetables for their β-carotene content by HPLC will help us to select the best sources of β-carotene, the question whether what is chemically available by the laboratory methods would be equivalent to biologically available provitamin A needs to be answered.

Thus, along with renewed efforts to obtain data on nutrient profile, the bioavailability of the provitamin A foods should also be appraised.
The highly seasonal nature of the carotene rich foods also calls for appropriate preservation so that they can be used during lean season (Sesahdri, 1996). In this context dehydration of the leafy vegetables have been studied in this laboratory (Seshadri et al, 1997), with good potential for retention of \( \beta \)-carotene (50%). However, there are no studies available on how well the \( \beta \)-carotene from dehydrated leaves is available.

Several methods are available for assessing the bioavailability of carotenes such as bioassays with rats as the animal model, dark adaptation tests and balance studies. Rats are standard animals employed for the vitamin A bioassays (Callison and Orient-Keiles, 1947). Vitamin A deficiency has been most extensively studied in the rat, which can be depleted of the liver stores before the bioassay is carried out (Sweeney and Marsh, 1973; Stoecker and Arnrich, 1973; Underwood et al, 1979; Davila et al, 1985).

The fourth section of this study thus addressed the bioavailability of \( \beta \)-carotene, in a rat model, from one of the richest sources of carotene identified in section I & II, namely drumstick leaves, in fresh and dehydrated forms.

PROCESSING LOSSES INADEQUATELY DOCUMENTED IN THE LITERATURE

Household processing of vegetables involves several steps such as washing, peeling, trimming, cutting, grinding and cooking by any one of the several methods, during which the foods maybe exposed to an acidic or alkaline pH, higher temperature and oxygen. Many of these have an adverse effect on the provitamin A content of the foods. The acidic pH can convert the trans to cis isomer, ultraviolet light and oxygen can convert the carotene into oxygenated derivatives and lipooxidas present in foods can generate free radicals that can destroy beta carotene (Labuza and Tannenbaum, 1972; Ranganath and Dubash, 1981; Park, 1987). Thus the carotene content of the
fresh vegetables may not necessarily be indicative of how much carotene is consumed.

Different methods of processing can cause varying losses of β-carotene. Recent studies have shown that some household processing methods such as merely chopping or raw grinding leads to minimal loss as compared to prolonged cooking after grating as done for carrot halwa which resulted in a loss of 80% of β-carotene (Reddy, 1995). Studies in our laboratory have shown that sautéing resulted in minimal losses of β-carotene from GLVs, while open pan cooking resulted in substantial losses (Pasham, 1995).

Data on losses of β-carotene, when the provitamin A foods are incorporated into a cereal-pulse mix have not been taken up. Many supplementary feeding programs in India, have the opportunity and scope to increase the carotene and hence vitamin A intake of the vulnerable groups if, the easily available plant foods rich in β-carotene can be incorporated into the cereal pulse, energy supplement that is routinely used in this setting (Devadas et al, 1979; Shehata and Fryer, 1970, Juneja, 1980; Rao et al, 1980; Smith, 1982; Katiyar et al, 1985).

Therefore, the last section of this study addressed the question of the maximum level of one of the underutilized leafy vegetables in this region, namely radish leaves, that could be incorporated into a cereal-pulse mix and the extent of retention of the β-carotene when the mix was subjected to several common methods of processing. The methods of processing selected were baking, steaming and shallow frying, which were also found to be commonly used by the community in this region.
OBJECTIVES OF THE STUDY

From the foregoing, the objectives of the present study may be summarized as follows:

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 by high performance liquid chromatography in seventeen of the above key carotene rich foods, all of them leafy vegetables.

3. To analyze the content of selected nutrients and antinutrients in these GLVs.

4. To select a leafy vegetable, that was a rich source of β-carotene and study its bioavailability in the fresh and dehydrated form.

5. To formulate recipes using an underutilized leafy vegetable, involving three methods of household processing and establish the retention of β-carotene in them.

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 last chapter VII summarizes the results of the study and outlines the major conclusions.