

Principal Investigator M. Mujibur Rahman Trainee Investigator (if any) _____
 Application No. 83-027P Supporting Agency (if Non-ICDDR,B) _____
 Title of Study A STUDY ON THE VITAMIN Project status:
CONTENT OF VEGETABLE BASED COOKED FOOD () New Study
 () Continuation with change
 () No change (do not fill out rest of form)
REQUESTED BY PEOPLE OF DIFFERENT SOCIO-
ECONOMIC GROUPS IN BANGLADESH

Circle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:

(a) Ill subjects Yes No

(b) Non-ill subjects Yes No

(c) Minors or persons under guardianship Yes No

Does the study involve:

(a) Physical risks to the subjects Yes No

(b) Social Risks Yes No

(c) Psychological risks to subjects Yes No

(d) Discomfort to subjects Yes No

(e) Invasion of privacy Yes No

(f) Disclosure of information damaging to subject or others Yes No

Does the study involve:

(a) Use of records, (hospital, medical, death, birth or other) Yes No

(b) Use of fetal tissue or abortus Yes No

(c) Use of organs or body fluids Yes No

Are subjects clearly informed about:

(a) Nature and purposes of study Yes No

(b) Procedures to be followed including alternatives used Yes No

(c) Physical risks Yes No *Not applicable*

(d) Sensitive questions Yes No *Not applicable*

(e) Benefits to be derived Yes No

(f) Right to refuse to participate or to withdraw from study Yes No

(g) Confidential handling of data Yes No

(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No *NA*

5. Will signed consent form be required:

(a) From subjects Yes No

(b) From parent or guardian (if subjects are minors) Yes No

6. Will precautions be taken to protect anonymity of subjects Yes No

7. Check documents being submitted herewith to Committee:

- Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
- Protocol (Required)
- Abstract Summary (Required)
- Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
- Informed consent form for subjects
- Informed consent form for parent or guardian
- Procedure for maintaining confidentiality
- Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:

1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

I agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Mujibur Rahman
Principal Investigator

Trainee

83-027P

28/6/83

SECTION I - RESEARCH PROTOCOL

1. TITLE: A STUDY ON THE VITAMIN A CONTENT OF VEGETABLE BASED COOKED FOOD INGESTED BY PEOPLE OF DIFFERENT SOCIO-ECONOMIC GROUPS IN BANGLADESH

2. PRINCIPAL INVESTIGATOR: M. Mujibur Rahman

CO-INVESTIGATORS: Mr. Akbar Ali
Mr. M.A. Wahed

3. STARTING DATE: July 1983

4. COMPLETION DATE December 1983

5. TOTAL DIRECT COST: US \$ 5965.00 (Incremental Cost \$ 2999.00)

6. SCIENTIFIC PROGRAMME HEAD:

This protocol has been approved by the Nutrition Working Group.

Signature of Scientific Programme Head: _____

A. Molla

Date: _____

22. 6. 83

7. ABSTRACT SUMMARY

Balanced nutrition is essential for good health. Unfortunately, the majority of our people live on food which falls far below the normal nutritional level compared to the developed societies. Reports are available on nutritional value, especially vitamin A content of different uncooked vegetables, but no data are available regarding vitamin A of different vegetable based cooked food items in Bangladesh. We have decided to estimate vitamin A content of leafy green and other vegetables before and after cooking. These data will show the intake of vitamin A in the village community of different SES. This investigation will also allow us to understand the amount of vitamin A lost during cooking procedures.

8. REVIEWS:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e. Controller/Administrator: _____

ABSTRACT SUMMARY

1. Altogether 450 raw and cooked vegetable samples will be analysed for beta-carotene estimation.
2. No risk of any kind is involved in this study.
3. Not applicable.
4. All records will be kept strictly confidential. They will remain with the Principal Investigator.
5. Not applicable.
6. Interview only related to their food storing and cooking procedure will be taken.
7. This study will help to determine the extent of loss of vitamin A in cooked vegetables.
8. Not applicable.

SECTION II - RESEARCH PROTOCOL

A. INTRODUCTION:

1. Objectives:

Vegetables containing pro-vitamin A are being taken by the rural people. Nevertheless, night blindness and other vitamin A deficiency diseases are common among them which partially could be due to faulty cooking of food. The present study will quantitatively estimate vitamin A in different vegetables before and after cooking.

2. Background:

The cooking procedure of various foods are quite different in this country from that of the developed countries. Sood and Bhat (1974) compared the traditional (Indian) method, in which vegetables are boiled in open vessels until the water content is evaporated, to other methods of vegetable cooking. The traditional method gave poor retention of carotene. It is anticipated that estimation of food values from cooked and uncooked foods will enable us to suggest the exact amount of vitamin A loss during cooking. This study may indicate the necessity of undertaking different methods of cooking of food to minimize loss of nutrients, especially vitamin A. With a per capita income of about US\$ 110.00 (WHO report, 1980), per capita consumption of cereal, vegetable and protein are at the lowest level in Bangladesh compared to developed countries. Poor nutrition has a major contribution to the high mortality rate of children upto three years of age, (Khan, M.U., 1981 - Sommer et al, 1975). Moreover, incidence of night blindness (vitamin A deficiency disease) is common in developing countries (Pirie, 1983).

In Bangladesh, 20-30% of the children die before their fifth birthday and about 0.8 million of our population suffer from night blindness due to vitamin A deficiency. It is also estimated that 1.3% children of the age group 0-4 years and 3.8% boys of the age group 5-14 years suffer from night blindness, (Nutrition Survey of Rural Bangladesh, 1975-76). Foods containing preformed vitamin A are expensive and are beyond the reach of the poor. But alternative less expensive sources of provitamin A are easily available through vegetables. Green leafy vegetables are rich sources of beta-carotene (Vinod and Reddy, 1979).

In this context, it is necessary to assess the vitamin A content in vegetable-based foods consumed by people of different socio-economic status. This will provide us the knowledge to understand the causes of vitamin A deficiency diseases, inspite of green vegetable consumption by most of the people. The average intake of vitamin A in Bangladeshi people is 714 I.U./person/day, which meets only 35 percent of their requirement. Vitamin A intake in the children of 1-3 years of age is only 253 I.U./person/day, which meets only 30 percent of their requirement. Children of 4-6 and 7-9 years of age get 50 and 44 percent of vitamin A requirement respectively (Nutrition Survey of Rural Bangladesh, 1975-76).

The chief nutritive value of vegetable is not only due to the presence of beta-carotene but also that of ascorbic acid and folate (Sir Stanely Davidson, et al, 1979). Average consumption of other vegetables per person per day is 134 gm and the consumption of leafy green is 15.8 gm. However, it is interesting to note that about 15-20 gm of vegetable containing high amount of beta-carotene is sufficient to the daily needs for vitamin A of a young child (Begum and S.M. Pereira, 1977).

In some studies (Vonod R. Lala & Vinodini Reddy, 1980, Nageswara Rao & Narasing Rao, 1970) it was shown that beta-carotene from vegetable based diet is absorbed to an extent of 54-70%. Previously, it was shown that loss of vitamin A in both Indian and western foods due to cooking is very insignificant, but they did not explain methods of cooking process, (Nutritive value of Indian foods, 1980 - Ethel Austin Martin, 1964). It was also stated that both retinol and beta-carotene are stable to "ordinary" cooking method, though some loss may occur at temperature above 100°C when butter or palm oil is used for frying. Fruits and other foods that are sun dried lose much of their vitamin A potency (Sir Stanely Davidson, et al, 1979). It has been reported that loss due to cooking could be minimized where processing time and temperature are kept low (Kenneth Simpson, 1983, - Rao and Reddy, 1974). So, it is possible that some of our cooking methods may destroy a good quantity of the nutrients and vitamins. In view of the above background, a study will be made to determine loss of vitamin A in cooked vegetable and to understand whether the remaining amount is enough for us.

Of the available information high beta-carotene content of commonly used Bangladeshi food items (leafy vegetables), the following is a comprehensive list showing the beta-carotene content (Nutrition and Food Science, 1980 - Nutritive value of Indian Foods, 1982).

	Edible green leaves with a high content of beta-carotene	Available Season	Beta-Carotene Content	
			Nutr. food Sc. μ gm/100	Nutr. value of Indian foods μ gm/100
1.	Dhaina Pata (Coriandrum Sativum)	Winter	6918	6918
2.	Palang Sak (Spinacea Oleracea)	"	8470	5580
3.	Mula Sak (Raphanus Sativus)	"	9700	5295
4.	Lal sak (Amaranthus Gangeticus)	"	11940	5520
5.	Khesari Sak (Lathyrus Sativus)	"	3000	3000
6.	Motor Sak (Pisum Sativum)	"	-	-
7.	Mistikumra Sak (Cucurbita Maxima)	"	12000	-
8.	Sarisa Sak (Brassica Campestris)	"	1998	2622
9.	Sarisa Sak (Brassica Napus)	"	-	-
10.	Pat Sak (Corchorus Capsularis)	Summer	11700	-
11.	Data Sak (Amaranthum Lividus)	"	10100	-
12.	Kalmi Sak (Ipomoea Reptans)	"	10740	1980

	Edible green leaves with a high content of beta-carotene	Available Season	Nutr. Food Sc. µ gm/100	Nutr. value of Indian foods µ gm/100
13.	Pui Sak (Basella Alba)	Summer	12750	5580
14.	Kachu Sak, green variety (Colocasia Antiquorum)	"	10278	10278
15.	Kachu Sak, black variety (Colocasia Antiquorum)	"	12000	12000
16.	Helencha Sak (Enthydra Fluctuan)	"	13700	-
17.	Kacha Morich (Capsicum Annum)	"	2300	175
18.	Shukna Morich (Capsicum Annum)	"	-	345

Units: Vitamin A activity in foods is expressed in international units (IU), 1 IU being equivalent to 0.3 µg of retinol, 0.344 µg retinyl acetate, 0.55 µg retinyl palmitate, 0.6 µg of beta-carotene or 1.2 µg of provitamin A carotenoids other than beta-carotene (WHO, 1976).

Incidentally, researchers in Bangladesh as well as in India have missed to evaluate the beta-carotene content in certain vegetables. Moreover, certain nutrients, especially beta-carotene, vary considerably from one vegetable to another such as pat sak (Corchorus Capsularis), data sak (Amaranthus Lividus), mistikumra sak (Curubita Maxima), kalmi sak (Ipomoea Reptans), lal sak (Amaranthus Gangeticus), motor sak (Pisum Sativum) etc. It is, therefore, necessary to repeat the estimation of these vegetables to determine beta-carotene content.

Cooking Procedure of Vegetables in Bangladesh.

Different cooking procedures as practiced all over Bangladesh are described as follows:

1. As rich is partly boiled, either one of the vegetables like potato (*Solanum Tubersum*), brinjal (*Solanum Melongena*), bean (*Phasedus Coccineus*), pumpkin leaves (*Curcurbita Mixima*), bitter gourd (*Momordica charantia*), or raw chillies (*Capsicum Annum*) are placed on the surface of the boiling rice. With the boiling of rice these vegetables too are boiled. These vegetables are then crushed with raw onion chips, salt and mustard oil to make a paste for consumption.
2. Tender leaves of any vegetable are chipped into small pieces and then washed. Then these are boiled for about 10 minutes and the fluid emanating from the boiled vegetables is either thrown away or allowed to evaporate. After evaporation, it is fried with green or dry chillies, oil, salt, onion and garlic before consumption. Some people, however, do not follow the last method, they add oil, salt and spices just at the time of boiling the vegetable. Sometimes, palang sak (*Spinacea Oriracea*) or pui sak (*Basella Alba*) or kachu sak (*Colocasia Antiquorum*) is cooked with dry fish, with fresh hilsa or with shrimps.
3. Niramish or mixed vegetables (more than two kinds of vegetables) such as potato (*Solanum Tubursum*), seem (*Dolichos Lablab*), papaya (*Carica Papaya*), barbati (*Vigna Catjung*), shajina (*Moringa Oleifera*), mistikumra (*Cucurbita Maximal*), karala (*Balsum Apple* or Bitter Gourd), etc. are cooked with salt, green chillies, onion and oil.
4. Vegetables like tomato (*Lycopersicon Esculentur*), potato (*Solaunum Tubursum*), papaya (*Carica Papaya*), data (*Amarunthus Lividus*) are also cooked with fish or meat.

These are the general procedure practised in most part of Bangladesh. However, they may vary from family to family depending on their socio-economic status, morbidity condition and other cultural factors.

B. SPECIFIC AIMS:

- a) To estimate the quantity of vitamin A in leafy green and other vegetables, immediately before and after cooking.

- b) To estimate the amount of vitamin A in cooked vegetables just before being ingested. This will tell us whether vitamin A is lost during the period, food was left standing at room temperature before ingestion.

C. METHODS:

The present study will be conducted in villages of Matlab area. The inhabitants are mostly cultivators and the major crops are rice (*Oryza sativa*), lentil (*Lens esculenta*), jute (*Corchorus capsularis*), sweet potato (*Ipomoea batatas*) and potato (*Solanum tuberosum*), mango (*Mangifera indica*), jack fruit (*Artocarpus heterophyllus*), guava (*Psidium guajava*) etc. are available seasonally in household gardens.

Kacha morich (*Capsicum Annum*), dhaina pata (*Coriandrum sativum*), onion leaves or plaj pata (*Allium cepa*), mula sak (*Raphanus sativus*), data sak (*Amaranthus Lividus*), pumpkin or mistikumra sak (*Curcubita Maxima*), sarisa sak (*Brassica campestris*), pui sak (*Basella alba*), pea or motor sak (*Pisum sativum*), kachu sak (*Colocasia antiquorum*) jute leaves or pat sak (*Corchorus capsularis*), are the popular seasonal green leafy vegetables.

The present study will be done in two parts. First of all a preliminary survey will be conducted to find out the methods of cooking vegetables in the village households of different socio-economic status (SES). SES is classified on the basis of education, occupation, total income, U.P. tax and ownership of articles (Stan D'Souza, et al, 1980). Separate proforma will indicate the same (see appendix). At least 10 samples from each group (i.e. 10 high, 10 middle and 10 from low income groups) will be taken to the laboratory for measuring the beta-carotene content.

500 gms of green leafy vegetables namely lal sak (*Amaranthus gangeticus*) or pat sak (*Corchorus capsularis*) will be supplied from local market to each family for cooking in their own way. Weight will be taken just before washing and cooking and 5.0 gms of uncooked vegetable will be collected for beta-carotene analysis. Once the cooking is over a second weight will be taken and 5.0 gms of samples will be collected for analysis of beta-carotene.

Cooking procedure will be recorded through observation with regard to washing, boiling time, water content, addition of oil and spices etc. A field worker will be trained to closely observe these procedures (see appendix).

Second Part: Three to four groups of samples will be collected in two main seasons namely in summer - rainy (June to September) and in autumn - winter (October to December) season.

Green leafy vegetables namely pui sak (Basella Alba), bhaita sak (Chenopodium Album), data sak (Amaranthus Lividus), pat sak (Corchorus Antiquorum), Helencha sak (Enhydra Fluctuan) are available in the summer season.

Vegetables like palang sak (Spinacea), mula sak (Raphanus Sativus), lal sak (Amaranthus Gangeticus), motor sak (Pisum Sativum), sharsa ful (Brassica Campestris), kacha morich (Capsicum Annum) are available in the winter season.

In the second part of the study, samples will be collected from 30 sites and given to families for cooking 10 from each socio-economic status. Selection of the families will be made according to random number.

Each item of the vegetables as selected by the study families will be weighed on a beam balance before and after cooking. 5 gms. of vegetables will be collected each time before and after cooking for analysis of beta-carotene.

This study will be repeated in each season (summer-rainy and autumn-winter) and will continue for six months late summer and early winter.

Method for Carotene Estimation:

(M. Holden, Rothamsted Experimental Station, Harpenden, U.K.)

1.0 gm of weighed sample of leaves is chopped quickly with sharp knife or scissors into a small mortar and then ground rapidly with sand and 5 ml acetone. Petroleum ether 5 ml is added and the grinding continued briefly. Most of the leaf pigments will now have been extracted. Now the extract is decanted into 25 ml volumetric flask. Three further extractions are made, each with about 4 ml petroleum ether, the extracts added to the flask and the volume made up to the mark. The flask, with all the pigments now in solution in petroleum ether containing 20% acetone, is kept in the dark until the next stage. If any debris has been carried over into the flask it should settle to allow a sample to be removed with a pipette. Usually, a 10 ml sample is taken so that the determination can be repeated if necessary. The carotene is separated from the other pigments on a column of Alumina.

Column:

Short column 2.5 cm in diameter with a sintered glass disc to hold the absorbent are ideal. Alumina i.e. alumina oxide active is mixed with equal weight of anhydrous sodium sulphate and the mixture is placed in the column to a depth of about 5 cm.

Assay:

The sample containing pigments is added carefully to the top of the column and when all the fluid has passed into the absorbent; petroleum ether is added. The carotene travels down leaving the other pigments behind and starts to come off in the first running of the column. Solvent is added until the elute comes off colourless. The elute is made up to known volume i.e. 20 ml and the absorbance is read on the spectrophotometer at 450 nm.

Carotene solution 2 ug/ml has an absorbance of 0.25 measured in a spectrophotometer.

D. RATIONALE AND SIGNIFICANCE:

So far, studies have been made on fresh vegetables in Bangladesh which indicate that the nutrient intake is fairly satisfactory, although some common vegetables have been left out from the survey. However, no data are available in this country in respect of cooked food including the varieties which are widely used extensively ingested vegetables.

Although international food tables inform us about the general pattern of nutrient contents of various foods, no detailed information is available in relation to our traditionally cooked foods.

The study will quantitate the loss of vitamin A due to cooking. It will also provide data and facts that can be correlated to vitamin A status nutrition with special reference to our food habits and cooking procedures.

E. FACILITIES REQUIRED:

Matlab clinical pathology laboratory will be utilized for over night storing of samples and ICDDR,B Biochemistry Laboratory will provide the facilities for vitamin A, carotene estimation.

F. COLLABORATIVE ARRANGEMENT

N i l

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SECTION III - BUDGET

A. DETAILED BUDGET

1. Personnel Services		% of	Annual	Dollar
<u>Name</u>	<u>Position</u>	<u>effort</u>	<u>Salary</u>	<u>US\$</u>
Mr. Mujibur Rahman	Principal Investigator	40		1646.00
Mr. Akbar Ali	Co-Investigator	5		290.00
Mr. M.A. Wahed	"	10		230.00
Research Officer	Biochemistry	20		300.00
Field Assistants (2)		50		<u>500.00</u>
				<u>2966.00</u>

2. Supplies and Materials				
a) Biochemistry tests :				
	Beta-Carotene in vegetables, 400			2000.00
b) Equipment: Weighing Balance (two)				
				600.00
c) Sample Collecting Container				
				10.00
d) Vegetables				
				25.00
3. a) Hospitalization				
				-
b) Out Patient Care				
				-
4. ICDDR,B Transport (speed boat run 50 hrs)				
				165.00
5. Road Transport				
				64.00
6. Transport of Materials				
				-
7. Rent Communication Utilities				
				-
8. Printing and Reproduction				
				90.00
9. Construction				
				-
10. Medicine				
				<u>45.00</u>
				2999.00
				<u>2966.00</u>

Total US\$				5965.00

SUMMARY BUDGET

A. Personnel	US\$ 2966.00
B. Supplies & Material	2635.00
C. Transport	229.00
D. Printing and Reproduction	90.00
E. Medicine	<u>45.00</u>
Grand Total	US\$ <u>5965.00</u> =====

Grand Total \$ 5965.00 - Personnel Cost = \$2966 = 2999.00

Incremental Cost US\$ 2999.00

SURVEY ON COOKING

1. a) House No. _____ Date _____ Field Assistant _____

b) Village _____ Thana _____ District _____

2. i) Family Head _____ ii) Age _____ Education _____ iv) Occup. _____

v) Income _____ vi) U.P. Tax Tk. _____ vii) Size of the House _____

viii) Made of _____ ix) Agr. Land _____ SES.- High/Middle/Low

3. Family Size _____ Male _____ Female _____ Children _____

4. Name of the respondent _____ Age _____ Education _____

5. Vegetables mostly used. Summer: a) _____ b) _____ c) _____ d) _____

Winter: a) _____ b) _____ c) _____ d) _____

6. Cooking Procedure :

Time of day : Morning _____ Noon _____ Evening _____

Name of vegetables	Wt. of raw veg. (gms)	Washing x times	Boiling Min.	Vessel open/close	Oil (gm)	Addition of spices	Speciality if any	Wt. cooked veg. (gms)	Remarks

7. Beta-Carotene of _____ ugm/100 gm (Expected Value)

Beta-Carotene of raw vegetable _____ ugm/100 gm Beta-Carotene of cooked veg. _____ ugm/100

সম্মতি পত্র

আন্তর্জাতিক উদ্বাসন গবেষণা কেন্দ্র জাইবিয়া চিকিৎসার সাথে
পুষ্টি সংক্রান্ত সমস্যা নিয়ে গবেষণা করে আসছে। আমাদের দেশের
শাক-সব্জি সে রয়েছে প্রচুর ডিটোমিন, যা আমাদের ডিটোমিন 'এ'র
উৎস তৈরিতে সক্ষম। উক্ত ডিটোমিন 'এ'র উৎস তৈরিত
স্বাস্থ্যকর ও স্বাস্থ্য দৃষ্টি সঠিক শ্রীতজ একটি বড় বরনের সমস্যা।
এ সমস্যার সমাধান জীবন প্রয়োজন।

আমরা, স্বাস্থ্য কল্যাণ ডিটোমিন 'এ' নর্থ হাফ সে সমস্যা
গবেষণা করতে চাই। আমরা চাই দেশ ও সমাজের স্বার্থে আপনি
এই গবেষণায় অংশ গ্রহণ করুন। আপনি যদি রাজি থাকেন
তা হলে :

- ১। আপনার পরিবারের অন্য ডিটোমিন 'এ' সমৃদ্ধ শাক দেখা
হবে। শাক রান্না করার পূর্বে ও রান্না করার পরে ওজন
করতে দেবেন ও রান্না প্রণালী সংক্রান্ত কিছু প্রশ্নাবলীর
উত্তর দেবেন।
- ২। অনুগ্রহ করে মাসে ৩ থেকে ৪ দিন আমাদের দেশ
শাক আপনার বাড়ীতে রান্না করবেন।

এই গবেষণায় অংশ গ্রহণে আপনার কোন বাধা বাবস্থা
নেই। আপনি আপনার সম্মতি যে কোন সময়ে প্রত্যাহার
করতে পারবেন।

আপনি যদি রাজি থাকেন তবে নীচে স্বাক্ষর করুন
কিংবা কাম থাকলে স্বাক্ষরের ছাপ দিন।

গবেষকের স্বাক্ষর
তারিখ

অংশ গ্রহণে শুরুর স্বাক্ষর
তারিখ

CONSENT FORM

International Centre for Diarrhoeal Disease Research, Bangladesh has been conducting researches on problems related to nutrition and diarrhoea. Although vegetables grown in our country contain significant quantity of vitamin which can adequately meet vitamin A requirement, night blindness or poor eye sight due to vitamin A deficiency is a major health problem in this country.

In this context, we want to study the loss of vitamin A during cooking. We would like you to participate in this research programme for the greater interest of the society. If you agree:-

1. We will supply you with vegetables rich in vitamin A. You will please allow us to take weight of the vegetables before and after cooking and answer some questions concerning the cooking method.
2. You will please cook vegetables supplied by us 3-4 times a month.

It is not obligatory for you to participate in this study. You are free to withdraw your consent at any time.

If you wish to voluntarily participate in this study, please sign or give left thumb impression below.

Signature of Investigator

Date _____

Signature or LTI of the participant

Date _____