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# Composition and Isolation of Beta Carotene from Different Vegetables and Their Effect on Human Serum Retinal Level

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**Abstract:** In this study we investigated the content of beta carotene in different kind of vegetables by HPLC. The vegetables were also analyzed for crude food constituents by standard methods of AOAC. The present study indicated that the vegetables are good source for beta carotene and other food constituents. The effect of beta carotene on serum retinal (vitamin A) level was also tested by feeding the vegetable containing the higher amount of beta carotene (spinach) as source of beta carotene. This also showed the importance of beta carotene by enhancing the level of beta carotene in the test subjects. Due to beta carotene content and other food constituents like moisture, crude fiber, ash, fat, NFE, energy and crude protein contents the vegetables have a right place in our daily diet.

**Key word:** Beta carotene · HPLC

## INTRODUCTION

Carotenoids are synthesized exclusively by photosynthetic organisms including crop plants, algae, few fungi and certain bacteria where they play a vital role in plant metabolism and bio-synthesis of other bio-molecules. [1]. The carotenoids are undoubtedly among the most widespread and important pigments in living organisms. This group of pigments is found throughout the plant kingdom (although their presence is often masked by chlorophyll) and in insects, birds and other animals. These pigments provide a whole range of light yellow to dark red colorings and when complexed with proteins, green and blue colorations are achieved. Thus, a wide variety of foods and feeds-yellow vegetables, tomatoes, apricots, oranges, egg yolk, chicken, butter, shrimp, lobsters, salmon, trout, yellow corn, etc. owe their color principally to carotenoids, as do certain food color extracts from natural sources such as palm oil, paprika, annatto and saffron [2].

Beta-carotene, the principal carotenoid in carrots, is a familiar carotene, while lutein, the major yellow pigment of marigold petals, is common xanthophylls [3]. Vitamin A, or retinol, is an essential micronutrient for humans and other mammalian species since it cannot be synthesized within the body. Deficiency of the vitamin results in adverse effects on growth, reproduction and resistance to infection. The most important manifestation of severe vitamin A deficiency (VAD) is xerophthalmia and irreversible blindness may eventually occur in one or both eyes. VAD is still an important micronutrient deficiency problem in many developing countries including Pakistan, afflicting large numbers of pre-school children. It is often associated with protein-energy malnutrition, parasitic infestation and diarrhoeal disease. The major functions of retinal in animals are its role in vision and in the maintenance of epithelial cell. The function of retinol in the retina is to serve as the precursor of 11-cis-retinal-dehyde, the chromophore of all known visual pigments [4].

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The most striking and extensive lesions caused by vitamin A deficiency are those affecting epithelial growth and differentiation. They are all defects of the outer and inner linings of the body. A basic tool in carotenoid and retinoid research and development activities is the content of these two groups of compounds in foods. In recent years, there has been particular emphasis on understanding the types and concentrations of various carotenoids in foods. It is thought that previously reported values of vitamin A activity in food composition tables may have been unreliable since methodologies were not standardized since reliable methods were not used. Advances in studies into the structure and properties of various carotenoids have shown that only a handful of the hundreds of carotenoids occurring in nature possess vitamin A activity. Some of these may occur in higher concentrations than β-carotene, the most potent precursor of vitamin A [5].

## MATERIALS AND METHODS

**Sample Collection for Beta Carotene:** Vegetables were collected from local markets in Peshawar from different shops. Each vegetable sample was taken and mixed together to get a composite sample. From composite sample, 100gm of lab sample was taken and from that, 10gm of sub sample were selected for extraction. The preanalyzed samples were washed with tap water and were kept in inert condition, at 4°C temperature.

**Pigment Extraction:** Carotene was extracted from vegetables by "Reversed Phased HPLC System" [6], of sub sample was homogenized in 30ml of acetone then 0.1% (BHT) solution and acetone was added as an antioxidant. The resulting extract was filtered through booknel's funnel. The residue was washed twice with acetone till it become colorless. The residue was discarded and the filtrate was combined with 20gm of anhydrous sodium sulphate. The anhydrous sodium suphate was removed through filtration and the volume of extract was reduced by rotatory evaporator. The extract was transfer quantitatively to 100ml volumetric flask and the volume was made up to the mark with acetone and water, so that the final extract contains 80% of acetone.

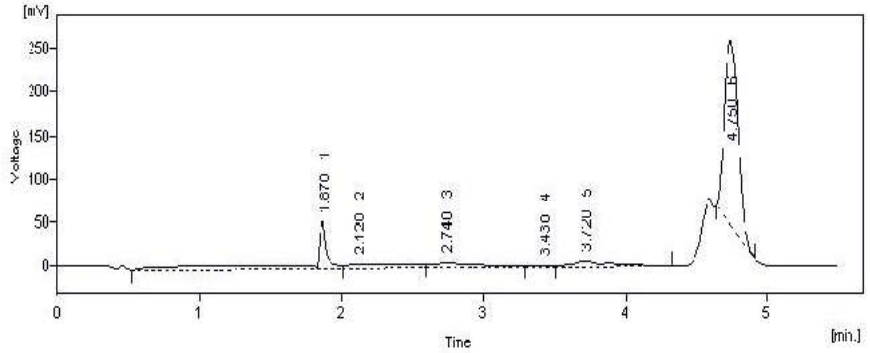
**Standard Preparation of Beta Carotene:** Solid standard of beta carotene (gm enclosed in vial) was obtained from Merck. 100ppm stock solution was prepared. 20ppm, 40ppm and 60ppm dilutions were made in 5ml of each acetone solutions. Perkin Elmer HPLC programme containing LC-1000 pump (Isocratic), having C18 column and connected with LC 250 UV/VIS detector was used. Peak identification and quantification was made by "CSW 32 software" for HPLC system. Wave length was fixed at 452 nm. The pressure of the column was kept 1800-2000 PSI.

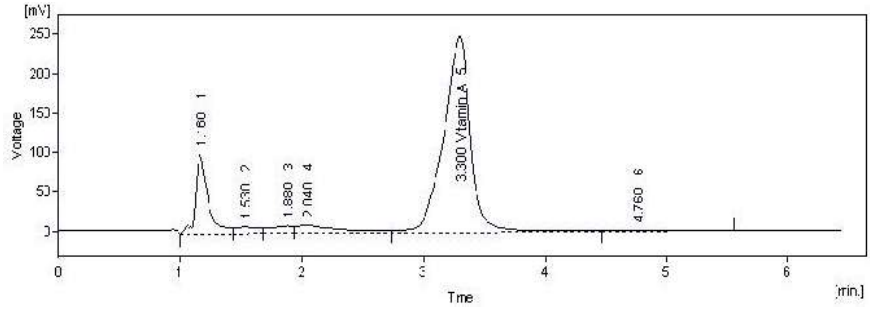
**Chromatogram of standard Beta Carotene**

**Sample Assay:** Each sample of beta carotene extract in 80% acetone was used for HPLC assay like standard; sample solution was taken by micro liter syringe. The peak was automatically identified and quantified by comparing its retention time of the sample with the standard retention time.

**Vitamin A Assay**

**Sample Collection:** 10 healthy humansubjects were selected voluntarily between ages 20 34 years. The serum was assayed before and after taking test diet (spinach = 240gm/ day) for a week. The test - diet was formed from spinach cooked in water with the addition of small amount of water, salt and spices. The cooking time and tenderness of the leaf was the same as usually practiced in homes. The diet was fed to each subject along with usual bread and water. The subjects were allowed for their daily routine activities. The subjects were on the same diet once in a day (at lunch time) for a week.






At the end of the feeding trial blood samples from each subject was taken in the morning time (7am) on 8th day before taking the diet.

**Extraction of Vitamin A from Blood:** The extraction and determination of vitamin A from blood serum were performed [7] with slightly modified to optimize the assay. Two hundred µl sera were taken into an eppendrof tube. Then 100µl ethanol was added. After thorough mixing, 400µl n-hexane was added. The mixture was again mixed well with vertex mixer for 1 minute and then the mixture was centrifuge at 12,000 rpm for 10 minutes. The upper solvent layer was collected and evaporated to dryness. The dried residue was dissolved with 100 µl ethanol.

**Quantification of Vitamin A:** Each sample extract of vitamin A (20µl) was injected when the injector was in load mode. HPLC was equilibrated by running mobile phase (methanol + water = 95:5, v/v respectively) at the rate of 1.5ml per minute with initial pressure of the column was kept at 1390-1500 PSI. Wave length was fixed at 452 nm. A distinctive sharp peak of vitamin A appeared in the chromatogram after 3.3 minutes (Rt = 3.3). The vitamin A content of serum was calculated from the standard curve.

**Chromatogram of Standard Vitamin A**

**Proximate Composition**

**Moisture:** Moisture was determined by oven drying method. The percentage moisture was calculated as:

% Moisture = W1 – W2 (100) / Wt of Sample.

**Crude Fat:** Crude fat was determined by Soxlet Extraction method using low boiling point ether as a solvent. Percentage crude fat was determined by using formula:

% Crude Fat = (Wt of beaker + ether extract) – (Wt of beaker) X 100 / Wt of sample.

**Crude Protein:** The protein content was estimated from nitrogen content determined by kjeldahl. 0.5-1.0 gm of sample was weighted and transferred to a micro kjeldahl digestion flask. The sample was mixed with 10-15 ml concentrated sulphuric acid and 8 gm of digestion mixture (potassium sulphate and copper sulphate). Small number of pumice stones was also added to avoid bumping of mixture during digestion. The flask was swirled in order to mix the contents thoroughly. It was heated till the mixture became clear and organic matter was oxidized to inorganic form. After digestion, the heater was stopped off and the flask was cooled. The digest was then transferred to 100ml flask. Distillation of the digest was performed by Markam still distilled apparatus [6]. About 10ml of digest was taken with the help of a pipette and was introduced in the volumetric flask and the volume was made up to the mark with distilled water. Then about 10 ml of NaOH was gradually added through the same way. Distillation was continued for at least 15 minutes and NH3 produced was collected in a conical flask containing 20 ml of 4% boric acid solution with few drops of methyl red indicator. The receiving water was kept cooled by ice water during distillation. The distillation was stopped when the pink color in the receiving flask became yellowish. The distillate was titrated against 0.1N HCl, till the appearance of pink color. The titration value was recorded and the protein value was calculated as follows:

% N = (S-B) x N x 0.014 x D x 100 / Wt of sample x V
S = Sample titration reading
B = Blank titration reading
N = Normality of HCL solution
D = Dilution of the sample
V = Volume taken for distillation
0.014 = mille equivalent wt. of Nitrogen
Protein % + 6.25* x % N
(*Factor for vegetables)

**Ash:** Standard ash was determined by straight ash method in a muffle furnace [8]. Clean empty crucible was placed in a muffle furnace at 600°C for an hour, then the crucible was cooled in a dessicator and the weight of the empty crucible was noted (W1). Dry sample (0.5-1gm) was placed in the crucible (W2). The difference between W1 and W2 gave the weight of the sample (W3). The sample was charred over the burner with help of blowpipe. The crucible was then placed in the muffle held at dull red heat

(550-600°C) for 2 to 4 hours. After complete ignition the furnace was turned off. The crucible was cooled and weighted (W4).

Percentage Ash was calculated as:
% Ash = Wt. of ash (W4 – W1) x 100 / Wt. of sample

**Fiber:** Crude fiber was determined by acid and alkali digestion method [6].The weighted sample was first digested with acid and then with alkali.

A known sample (1gm) was taken in a clear beaker and 100ml of 2.5% HCl was added to it. The mixture was boiled with stirring for about half an hour. It was then filtered through linen cloth and the filtrate was washed with hot distilled water and organic solvent (alcohol). Residue was quantitatively transferred to another beaker for alkali digestion. The Lenin cloth was washed by using wash bottle and traces of any fiber over it was drained in to the beaker.

The fiber residue was again digested in 2.5% NaOH in a similar manner like that of acid digestion. The residue so collected was transferred to a dried crucible to remove moisture. Then the weight of the dry crucible was recorded. The crucible was then kept in furnace for red dull heat till the formation of white and grey ash. The crucible was cooled in dessicator and weighted again. The loss in weight of the dry residue upon ignition was taken as the amount of crude fiber. Percentage crude fiber was calculated as:
% Crude Fiber = (W1 – W2 x 100 / Wt. of sample) – F

**Nitrogen Free Extract (NFE):** Nitrogen free extract represents the digestible carbohydrates and can be found by the difference. This value was obtained by the subtracting the sum of the percentages of moisture, crude fiber, crude protein, crude fat and ash from 100.

NFE = 100 - % (Moisture + Crude Fat + Crude Protein + Crude Fiber + Ash)

**Food Energy:** The calorific value of the sample was calculated from the energy yielding components (Fats, Proteins and Carbohydrates).

## RESULTS AND DISCUSSION

The composite samples of different kinds of vegetables available at local markets in Peshawar were analyzed for their beta carotene content using High Performance Liquid Chromatography (HPLC). Proximate composition of these samples was also determined by standard method of AOAC (1990). To test the effect of carotene on blood serum retinol level, carotene rich diet (spinach = 240 gm/day) was fed to ten volunteers for one week and the serum was analyzed for retinol by HPLC. The data concerning the beta carotene content of vegetables is given in Table 1. Serum retinol content of 10 human subjects fed the above test diet is shown in Table 2, while proximate compositions of vegetables are given in Table 3.

Table 1: Beta carotene content (µg/100g and IU) in different fresh vegetables

| Sample | Local Name | Botanical Name | µg/100g | IU |
|---|---|---|---|---|
| Bath spongy | Tori | *Lufa sagyptice* | 966 | 579 |
| Bitter Guard | Karila | *Monordicacharentia* | 1078 | 646 |
| Bottle Guard | Khadu | *Legennaria vulgarus* | 140 | 84 |
| Bringal | Bangan | *Solanum melongena* | 2100 | 1200 |
| Cabbage | Bhand Gobi | *Brasicca capitata* | 910 | 546 |
| Carrot | Moli | *Daucasa carota* | 11210 | 7266 |
| Cucumber | Khira | *Cucumus sativus* | 280 | 168 |
| French beans | Kacha Lobia | *Vicia faba* | 882 | 529 |
| Green Chili | Sabaz Mirch | *Citrulus fistulosus* | 1750 | 291 |
| Khulfa | Sag | *Capsicum annum* | 6580 | 3948 |
| Lady finger | Bhindi | *Hibiscus esculentus* | 3220 | 1932 |
| Lettuce | Salad | *Lactuca sativum* | 3220 | 1932 |
| Mushrooms | Khubi | *Agricus compampestris* | Tr | - |
| Mint | Podina | *Menthe viridus* | 4550 | 2730 |
| Mountain ebony | Kachnar | *Bauhinia variegate* | Tr | - |
| Onion | Piyz | *Allium capa* | Tr | - |
| Potato | Alu | *Solunum tuberosum* | Tr | - |
| Red chelli | Surkh Mirch | *Citrulus fistulosus* | 3290 | 1974 |
| Spinach | Palik Sag | *Spanacia oleracea* | 9940 | 5940 |
| Tomato | Timater | *Lycopersicum esculentum* | 1610 | 966 |
| SDV | | | ±2038.57 | |

Table 2: Serum retinol level (µg/dl) of the individuals before and after carotene based diet

| Sample | Before (µg/dl) | After (µg/dl) |
|---|---|---|
| A | 6.7 | 19.4 |
| B | 14.1 | 16.2 |
| C | 22.1 | 40.5 |
| D | 35.0 | 55.4 |
| E | 17.0 | 30.7 |
| F | 32.8 | 38.5 |
| G | 10.8 | 25.6 |
| H | 22.9 | 68.7 |
| I | 15.0 | 40.3 |
| J | 20.3 | 35.6 |
| Mean | 19.6 | 37.1 |
| SDV | ±9.01 | ±15.92 |

Table 3: Proximate composition (g/100g) in different fresh vegetables

| Sample | Local name | Botanical Name | Moisture | Fats | Protein | Fiber | Ash | NFE | Food |
|---|---|---|---|---|---|---|---|---|---|
| Energy | | | | | | | | | |
| Bath Sponge | Tori | *Luffa segyptice* | 93.3 | 0.2 | 1.1 | 1.0 | 0.4 | 4.0 | 22 |
| Bitter gourd | Karila | *Monordicacharentia* | 92.1 | 0.2 | 1.4 | 1.3 | 0.7 | 4.4 | 25 |
| Bottle Gourd | Kadu | *Legenaria vulgarus* | 94.1 | 0.2 | 1.0 | 0.7 | 0.5 | 3.5 | 22 |
| Bringal | Bangan | *Solanum melongena* | 91.7 | 0.2 | 1.3 | 0.9 | 0.4 | 5.5 | 29 |
| Carrot | Gajar | *Beta vulgarus* | 88.4 | 0.2 | 0.9 | 0.8 | 0.7 | 9.0 | 41 |
| Coriander | Dhania | *Coriandrum sativum* | 99.0 | 10.9 | 15.2 | 3.2 | 5.0 | 27.8 | 110 |
| Cucumber | Khira | *Cucumis sativus* | 90.2 | 0.2 | 1.8 | 1.0 | 0.6 | 6.1 | 33 |
| French Beans | Taza Lobia | *VICIA FABA* | 70.2 | 0.8 | 8.8 | 19.0 | 12 | 17.1 | 240 |
| Kulfa | Sag | *Portulaca oleracea* | 93.6 | 0.2 | 1.2 | 0.4 | 1.2 | 3.4 | 20 |
| Lady Finger | Bhindi | *Hibiscus esculentus* | 89.1 | 0.2 | 2.2 | 1.3 | 1.0 | 6.2 | 35 |
| Lettuce | Sag | *Lactuca sativum* | 94.4 | 0.3 | 1.4 | 0.5 | 0.8 | 25 | 108 |
| Mint | Podina | *Menthe viridus* | 87.5 | 0.5 | 2.9 | 1.4 | 1.6 | 6.1 | 40 |
| Mountain Ebony | Kachnar | *Bauhinia variegate* | 80.5 | 0.2 | 1.6 | 1.1 | 1.0 | 15.5 | 70 |
| Mushroom | Khubi | *Agricus campestris* | 93.1 | 0.2 | 3.2 | 0.6 | 0.6 | 23 | 106 |
| Potato | Alu | *Solunum tuberosum* | 76.7 | 0.2 | 1.8 | 0.4 | 0.7 | 20.2 | 89 |
| Red Chili | Surkh Mirch | *Capsicum frutescens* | 90.0 | 0.3 | 1.6 | 1.8 | 0.6 | 5.7 | 32 |
| Spinach | Palik Sag | *Spanacia oleracea* | 91.1 | 0.4 | 2.3 | 0.8 | 1.8 | 4.1 | 31 |
| Sweet Pipper | Shimla Mirch | *Capsicum annum* | 92.7 | 0.2 | 1.2 | 0.8 | 0.9 | 4.1 | 23 |
| Tinda | Tinda | *Citrulus fistulosus* | 93.1 | 0.1 | 1.9 | 0.7 | 0.6 | 3.6 | 23 |
| Tomato | Timater | *Lycopersicum esculentum* | 93.3 | 0.2 | 1.3 | 0.6 | 0.6 | 3.9 | 22 |
| SDV | | | ±201.8 | ±2.38 | ±3.39 | ±40.06 | ±2.64 | ±9.15 | ±180 |

Vegetables were analyzed for their beta carotene content because it is the precursor of vitamin A and eaten in both raw and cooked form by humans in daily life. Interest in beta carotene content of vegetables also generated due to its anti oxidant activity and anti carcinogenic characteristics[3]. The beta carotene content varied from trace amount in potato, mushroom and mountain ebony to thousands µg/100g in carrot, spinach, mint and lettuce. The present data were evident that dark green vegetables contained more beta carotene as compared to other vegetables. e.g. spinach contained 9940 µg/100g, followed by mint, kulfa, lettuce and lady finger that were all dark green in appearance, apart from carrot that contain maximum amount of beta carotene (11210 µg/100g) and which also responsible for the name of this compound [1]. These observations fairly agreed to the data followed by (Williams*et al*, 1996) in which beta carotene content in carrot was 12110 µg/100g, spinach 10230 µg/100g, lettuce 4787 µg/100g and tomato 1370 µg/100 g, they also reported trace amount of beta carotene content in onion potato and mushroom. The beta carotene content reported in this study was higher than that of who found 520 µg/100g in lady finger. This variation in beta carotene content of lady finger may be due to

varietals difference. The result of this study was also supported by [9], who analyzed 24 green vegetables for different micro nutrients contents including beta carotene.

They reported that beta carotene content in green vegetables ranges from 80-9204 µg/100gm. Like wise, beta carotene content of these vegetables also resembles with [10]. However, they reported much lower content of beta carotene in carrot (5340 µg/100g). In contrast, they reported much higher content of beta carotene in tomato (3500 µg/100g). It was obvious that, their samples of carrot may have higher moisture content as compared to those analyzed in the present study and the variation in tomato content of beta carotene may be due to the use of immature samples, because content of beta carotene drops by 77% during the ripening process, [11].

The slight variation in the data compared to others may be due to different in experimental condition such as extraction process, column length, diameter, temperature and different solvents used as mobile phase in HPLC etc. Cautious extraction and filtration process are needed for good results. Temperature during analytical process, the storage time of vegetable and extraction process of the samples must be kept in control because all these things has a tremendous effect on the results [12]. Variation in ecological growth conditions like variety and environmental aspects may also be contributing factors.

In order to asses the conversion of beta carotene to vitamin A in human body, sera of 10 adult human subjects, who were fed the richest carotene vegetable were assayed. The data showed in Table-2 reveals that average vitamin A content of sera varied from 19.67 to 37.09 µg/dl in blood before feeding the test diet. After feeding the test diet, the serum retinol content was increased in majority subjects. Considerable variation was noted among the various subjects in response to the same dose of carotenes derived from test diet. In 4 subjects there was marked increase in the sera retinol content, while in 5 subjects though the increased was not pronounced, however they showed an increased in trend of blood serum retinol. One of the subjects showed no response to the conversion of beta carotene to vitamin A; may be due to the physiological or pathological condition, which embedded the conversion of beta carotene to vitamin A. it was worth to mention that no subject complained about the adverse effect of the diet. The subject remained healthy during the experimental period and afterwards. This indicated that carotene has no side effect on human health, rather as a source of vitamin A the diet was protective and healthy.

The data of this study are in fair agreement with those of [5], who conducted similar type of experiment and observed some what similar results. In agreement with this study he also recorded considerable inter individual variation in bloods retinol level after administering oral dose of carotene rich diet in the form of powered algae (approx 135mg) [4] also supported the observation of the present study. They studied carrot as a source of alpha and beta carotene in 17 adult human subjects for a week and then examined their serum retinol content.

The results showed that carotene conversion to retinol is dependent on the physiology of individual. Because various enzymes are involved in the process of carotene retinol conversion and these enzymes might be varied from individual to individual. The conversion might also depend on the need of retinol to the body, the greater the need, the greater the conversion and so would be the level of serum retinol. As retinol is stored in the liver, when needed in other parts of the body, the liver attaches vitamin A to other parts of proteins, retinol binding protein (RBP), which is in turn attached to pre-albumin, both of which liver synthesis, if the liver is unable to synthesis these proteins, then vitamin A cannot be removed from the liver. Vitamin A as such is not lost from the body, but metabolites of vitamin A are excreted by way of bile into the feces. The person who ate more fatty diet with retinol source, his retinol demand is less, from plant source and apparent level of blood serum retinol would be lower.

The vegetables tested for beta carotene and vitamin A were also analyzed for their proximate composition/The data indicating moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract (carbohydrates) contents are presented in Table-3. The moisture content among vegetables was varied from 70.2 g/100gm in French beans to 93.3g/100gm in tomato. This reflected that the dry matter content in these vegetables was from 6.7g/10gm to 30g/100gm. Considerable variations existed among different vegetables with respect to their protein content (n = 6.25), which varied from 0.9 gm/100gm in carrot to 15.2 gm/100gm in coriander. Carrot contained minimum amount of protein, while coriander was the richest source of crude protein. Mushroom, spinach and lady finger were moderate source of protein. The crude fat was also variable and ranged from 0.1 g/100gm in tinda to 10.0 g/100gm in coriander. The crude fiber content of the dry matter of these vegetables was the lowest (0.4 gm/100gm) in kulfa and highest (3.2 gm/100gm) in french beans. Some vegetables like coriander, red chili and lady finger

were fairly good source of fiber, while potato, mushroom and bottle gourd were the poor source of crude fiber. The ash content was markedly different. The least amount was recorded in bringal (0.4 gm/100gm) and highest amount was noted in french beans (12.0 gm/100gm). Like ash variability also existed in nitrogen free extract (carbohydrates like starch, disaccharides and trisaccharides). Bath sponge contained lowest amount (4.0 g/100gm) of NFE, while maximum amount (27.0 g/100gm) was noted in coriander.

The proximate compositions of vegetables in this study were fairly fall in between the range of values reported by [13]. They analyzed selected vegetables for their proximate comp0ostion and mineral content, in which the moisture, crude protein, crude fat, crude fiber, ash and NFE were range form 70 to 90%, 0.2 to 14.2%, 0.2 to 0.5%, 3.0 to 18.2%, 0.5 to 0.9% and 5.0 to 25.2%, respectively. The present study was also in line with [14]. They analyzed several leafy vegetables for their proximate composition. Only the content of fiber was lower than the present study. Itwas cleared that, their samples had much higher moisture content as compared to those analyzed n present study.

The data was partially close with the work of [15], who analyzed the content of green vegetables. They reported much higher content of crude protein in lady finger (0.8 %) and mushroom (0.6%). This difference could be due to climatic and experimental conditions.

The present study was in disagreement with that of [16]. They analyzed wild vegetables for micronutrient content that showed much lower fat content.

Proximate composition provided general information about the purity and quality of food stuffs that helping in the formulation of balance diet and establishing legal standard for natural and stable vegetables.

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