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Comparative Study for the Extraction of β-Carotene in Different Vegetables

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Abstract: Beta carotene is a precursor of Vitamin A and is abundantly found in different vegetables and possess significant anti-oxidant potential. In the present study an attempt was made to determine the concentration of β-carotene from different vegetables sources viz carrots, corn, paprika and pumpkin. After the extraction its concentration was quantified by using spectrophotometer and the data was statistically analyzed. The results expressed that corn had highest amount of β-carotene (31%) followed by paprika (25%) then carrots (24%) and pumpkin (20%). This study provides a base line for extraction of costly β-carotene from local vegetable that can overcome the problem for the import β-carotene. Other findings revealed significant potential of this compound from indigenous sources and also found cheaper than the synthetic sources.

Key words: Beta carotene, vegetables, comparative study

INTRODUCTION

Hydrocarbon carotenoids are classified as carotenes, derived from the Latin name "carrots". Approximately 50 carotenoids known as "provitamin A compounds" considered as the precursor for retinol, an active form of vitamin A. Among the commonly occurring carotenoids such as β-carotene (alpha carotene), β-carotene (β-carotene) and lycopene, β-carotene is one of the most commonly occurring arotenoids (Sergio and Russell, 1999).

Beta-Carotene is being used often in food industry as food additive and are the most suitable formulation for many foods preparations. It is oil-soluble but can be miscible into a water-dispersible emulsion. The amount added in the food product depends on color shade desired but generally lies between 3-4 g pure β-carotene per ton. No restrictions have been placed on the level of use and it is listed as GRAS (Generally Regarded as Safe). Added β-carotene gives excellent stability in food products during processing and storage (Bauernfeind, 1981).

The final product of carotene in the living system is the compound retinol, which plays a key role in vision. The richest sources of β-carotene are yellow, orange and green leafy fruits and vegetables (such as carrots, spinach, lettuce, tomatoes, sweet potatoes, broccoli, cantaloupe and winter squash). In general, the amount of β-carotene present in the fruits and vegetables is directly proportional to the intensity of their color (Chandler and Schwartz, 1998).

Being an important antioxidant β-carotene protects cells from damaging effects of free radicals, due to its high radical scavenging activity (Fujisawa *et al*., 2004). It enhances the functioning of immune system, reduces the chances of cancer and heart diseases and a precursor of vitamin A. Vitamin A being very important vitamin and is also related to growth and differentiation of epithelial tissues in the body. In addition to the numerous studies on β-carotene's effectiveness for heart disease and cancer, researchers have been exploring its potential as a remedy against Chronic fatigue syndrome, Alzheimer's disease, fibromyalgia, male infertility and psoriasis.

Initially the researchers considered no meaningful difference between natural and synthetic β-carotene. This view was questioned when the link between β-carotene containing foods (all natural) and lung cancer prevention was not duplicated in studies using the synthetic pills.

In smokers, synthetic β-carotene has apparently caused an increased risk of lung cancer and disease of the blood vessels in double-blind research (Ben-Dore *et al*., 2005). Animal research has also identified that synthetic β-carotene might cause damage to lungs, particularly when animals are exposed to cigarette smoke. Increasingly, doctors are recommending that people supplement only with natural β-carotene (Myung *et al*., 2010).

Vitamin A deficiency is considered as a wide spread public health concern amongst pre school children in

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developing countries. Scale study for the nutritional problems in Pakistan showed that majority of the children in the study group was suffering from vitamin A deficiency. In recent UNICEF report 10-20% of children under the age 5 and 5.9% young mothers are suffering from vitamin A deficiency in Pakistan has the highest infant mortality and under 5 mortality (83.3 and 110/1000) in region with strong relationship to malnutrition (UNICEF, 2006). Beta-carotene as natural colors instead of synthetic colors also help to fulfill vitamin A deficiency that is especially found in Pakistan and it also decreases the chances of other diseases which are associated with artificial colors (Khan, 2002). Keeping in view the importance of this functional component in food the present study was designed to determine the amount of β-carotene from different vegetable sources and their extraction rate.

MATERIALS AND METHODS

The samples of corn, paprika, carrot and pumpkin were purchased from the local market and were shifted to the Department of Food Technology, Pir Mehr Ali Shah (PMAS) Arid Agriculture University, Rawalpindi (Punjab, Pakistan) where the project was executed. These were washed with distilled water, graded and than shredded and sliced aseptically.

Preparation of standard curve: Twenty five milligram β-carotene was dissolved in 2.5 mL chloroform and volume was made up to 250 mL with petroleum ether and 10 mL of this solution was diluted to 100 mL with petroleum ether. 5, 10, 15, 20, 25 and 30 mL of this solution was taken in volumetric flask each containing 3 mL of acetone. The concentration was 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 micro grams (µg) of β-carotene/mL after serial dilution. Absorbance recorded as Optical Density (O.D) at 452 nm was plotted against the β-carotene concentration (Fig. 1).

Extraction of β-carotene: Different extraction procedures were carried out for different vegetable samples. The extraction of β-carotene from corn (cv. Sahiwal, 2002) was carried out by the method described by Schaub and Islam (2004). For paprika (cv. Bell boy) method described by Ben and Fisher (1989) was used. On the other hand β-carotene extraction from carrots (cv. Autumn king) was carried out according to the method described by Ranganna (1997). However the Extraction for pumpkin (cv. Daisy petha) was done according to the method of Seo *et al.* (2004).

Preparation of column: Ten gram of silica was dissolved in the 100 mL of organic solvent i.e., petroleum ether (Scharlau, Barcelona Spain). In 20 mL syringe, a cotton wad was fixed in the bottom and then the column was packed with silica. After the preparation of column the extract was allowed to pass through it.

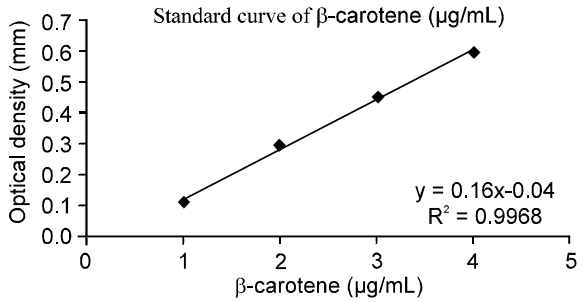

Fig. 1: Standard curve of β-carotene (µg/mL)

Adsorption and elution: It was done according to the method described by Ranganna (1997). Briefly, column was wetted by washing with 25-50 mL petroleum ether when the last ml of petroleum ether was still above the silica, vacuum was disconnected and adsorption column was transferred to clean dry flask. An aliquot (5-10 mL) of the extract to be chromatographed was columned. Successive washing of column with the eluent was carried out till β-carotene moved off the column and the eluent became colorless. Contents were transferred to the flask and diluted to volume with the eluent.

Spectrophotometric analysis: CE-2021, Spectrophotometer (CECIL Instruments Cambridge, England) was used to record Optical Densities (O.D) for different samples. An aliquot of petroleum ether extract of the sample was taken in to 100 mL volumetric flask containing 3 mL of diethyl ether and diluted to mark with petroleum ether. Absorbance was recorded at 452 nm.

Determination of β-carotene concentration: The calculations were done for paprika, carrot and pumpkin according to the equation described by Ranganna (1997):

$$\mu g - Carotene/gm = \frac{Conc.\,from\,SC(\mu g/mL)\,x\,FV\,x\,Dilution}{Weight\,of\,sample}$$

Where: SC: Standard curve, FV: Final volume

In case of corn, Lambert-Beer equation as described by Schaub and Islam (2004) was used to determine β-carotene.

$$A = E \times C \times D$$

where, A = Absorbance, E = Extinction molar coefficient, C = Concentration, D = Distance of light passed through solution.

Concentration of β-carotene was calculated as:

$$C = A/ED$$

Extinction molar coefficient for β-carotene is 134000 L/mol.cm.

Statistical analysis: The data obtained as the mean of three replications was analyzed in Complete Randomized Design using MSTATC Software as described by Steel *et al.* (1996).

RESULTS AND DISCUSSION

Pigments were extracted from corn, carrots, pumpkin and paprika and then quantified, the data regarding the comparison of β-carotene in these vegetables revealed that quantity of pigments extracted varied from one vegetable to other. Table 1 showed that all the results were highly significant ($p<0.05$). Corn stands first in ranking and showed the maximum amount of β-carotene in the recent study followed by paprika. Results also described that there was no significant difference between pumpkin and carrots and hence ranked on the same status. The results of present investigation were quite different from estimated amounts of β-carotene mentioned in previous studies except for pumpkin. It was also observed that the amount described in present study in each vegetable was quite in accordance with the a statement of Ranganna (1997) that extracted amount of beta carotene from vegetables or fruits can't be greater than 3 µL/mL. Beta-carotene was extracted from corn and then quantified by spectrophotometer.

Corn: Figure 3 further revealed that the β-carotene estimated from the corn sample in the present study i.e., 8460 µg/100 gm which was higher as compared to the amount 4288 µg/100 gm described by Schaub and Islam (2004). The calculated amount 2.4 µgm/mL in corn was plotted on standard curve of β-carotene (Fig. 4). This variation may be attributed to genetic and environmental factors like, as phenotypic of the corn used in this study was of deep orange color and may have large quantities of β-carotene as compared to yellow corn commonly grown in Europe for research and consumption. In addition the environmental factors may be an important reason of high β-carotene extraction in corn other then vegetables. The possible reason is soil nutrient profile since it requires large amounts of essential minerals, with substantial soil fertility level accumulating large nutrient contents in the final product. Similar views are expressed by Shanmugawelu (1985). Thus it was eventually deduced that corn requires high fertile soil for its proper growth and high production of β-carotene is favored by the soils having large quantities of nitrogen. Similarly stage of harvesting also affects the amount of β-carotene produced as it masked by the green pigment chlorophyll during the early stages of crop growth and its amount increases gradually with the progress in maturity (Kader, 2002). Vegetables stored in cool dark environment for specific time enhances the amount of β-carotene, corn used in present studies was stored for two months that was

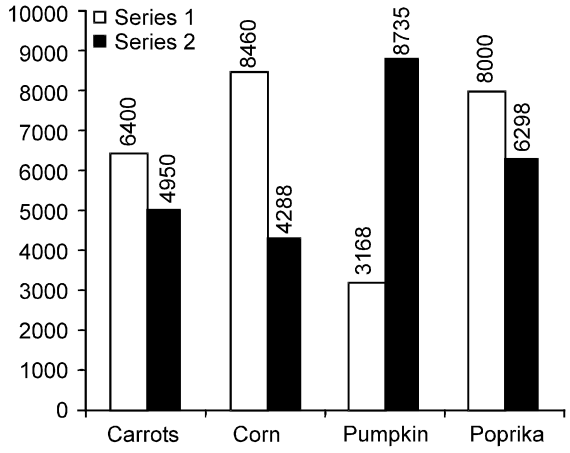

Fig. 2: Bar Chart showing the amounts of β-carotene (µg/100 gm). (Series, 1) Amount calculated in the present study and (Series, 2) Amount described by previous results

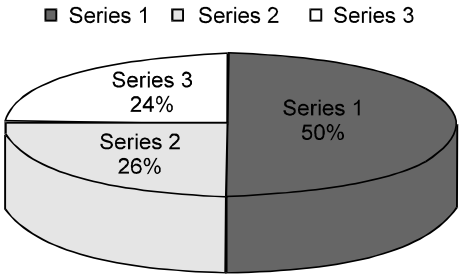

Fig. 3: Comparative detection of β-carotene (µg/100 g) in Corn. (Series, 1) Amount calculated in this study, (Series, 2) Amount described by Schaub and Islam (2004) and (Series 3) Amount described by Ranganna (1997)

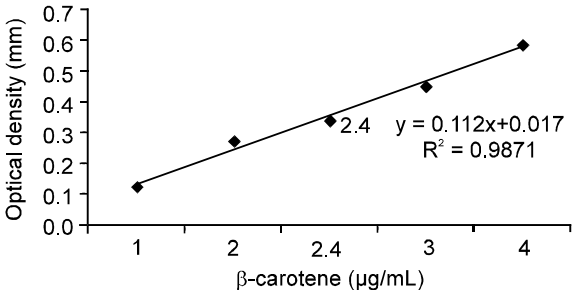

Fig. 4: Concentration of β-carotene (µg/mL) in corn

used for β-carotene extracted. These results negate the findings of Schaub and Islam (2004), who used fresh harvested corn for extraction of β-carotene The other reason for the variation may be the purity of chemicals used for extraction and lab conditions.

These results further revealed that there was a significant difference ($p<0.05$) in the concentration of

Table 1: Comparison between different vegetables based on β-carotene

Varieties	β-carotene (g 100 g)*
Corn (Sahiwal 2002)	0.3197 A
Paprika (Bell boy)	0.2913 B
Carrots (Autumn king)	0.2620 C
Pumpkin (Daisy petha)	0.2453 C

*Means with same letters are non significant at a-0.05 (LSD value: 0.01883)

Table 2: Estimated cost of extraction of β-carotene

Vegetables	Recovery of extract µg/100 gm	Cost/ extract Rs/µg	Fixed cost Rs/µg	Total cost Rs/µg	Cost of pigment Rs/Kg
Corn	8460	18	6	24	2500
Paprika	8000	14	6	20	2600
Carrots	6400	15	6	21	3300
Pumpkin	3168	14	6	20	6200

β-carotene among corn, paprika, carrots and pumpkin. The possible reason may include the promoters that are responsible for gene expression. Genes for β-carotene present in vegetables other then corn may have promoters but the promoter strength is less as compared to the promoter strength for beta carotene gene present in corn. So, the expression for β-carotene gene in other three vegetables may be less, as compared from to expression for β-carotene gene present in corn plant (Ben-Dore *et al*., 2005). Regulatory proteins in corn might bring variations and favored the production of β-carotene as compared to other three vegetables (Manitatis, 1989). Genes has various gene activator proteins bound to its regulatory region. However, these bound proteins are not sufficient on their own to activate transcription efficiently, the glucocorticoid receptor completes this combination. This combination may favor the high production of β-carotene in corn as compared to other vegetables (Albert, 1998). Corn is a warm season annual crop whereas carrots and paprika are winter season crops. Moreover, β-carotene production is favored greatly by high light intensity so being a warm season crop corn gets maximum light intensity and produce large amounts of β-carotene as reported by Shanmugawelu (1985).

Paprika: Estimation of β-carotene in paprika was carried out spectrophotometically at absorbance 452 nm as described by Ranganna (1997). Figure 5 expressed the data regarding the estimation of β-carotene from paprika revealed that quantity of β-carotene estimated in the recent studies was 8000 µg/100 gm that was much high as compared to estimated by Ben and Fisher (1989) that was 6298 µg/100 gm. The calculated amount 2 µgm/mL in paprika was plotted on standard curve of β-carotene (Fig. 6).

The data (Fig. 5) revealed that the amount calculated was greater as compared to amount described in previous studies by Ben and Fisher (1989) and

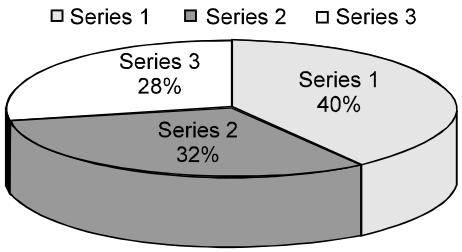

Fig. 5: Comparative detection of β-carotene (µg/100 g) in Paprika. (Series, 1) Amount calculated in this study, (Series, 2) Amount Described by Ben and Fisher (1989) and (Series, 3) Amount described by Ranganna (1997)

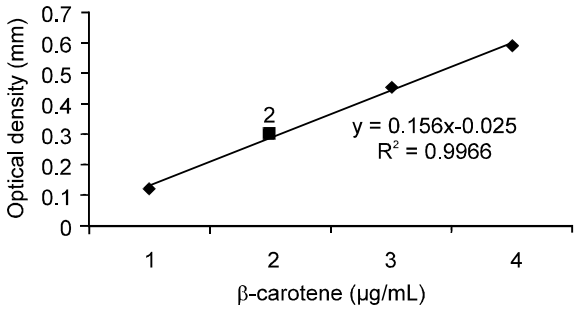

Fig. 6: Concentration of β-carotene (µg/mL) in Paprika

Ranganna (1997). The possible reasons may include varietals differences, storage conditions, lab conditions and reagents, along with methods of extraction used for β-carotene. In addition the soil fertility, agronomic practices and topographical factors may also affect the amount of β-carotene produced in a vegetable or fruit. It has been reported that the same varieties grown in different areas with different soil profile have different amount of pigments. High yield of paprika depends on high temperature, low moisture availability and high light intensity, so the difference in climatic conditions in different regions may bring the difference in the concentration of β-carotene produced by same vegetable which is vetted in regions of different climate. Paprika has a long growing season and therefore requires a judicious use of manures and fertilizers. Good fertile soils supplied with humus are most desirable for growing paprika. Good fertile soils may favor the production of β-carotene in paprika (Shanmugawelu, 1985). Like corn, paprika requires high nitrogen applications for its proper growth and yield and thus has high amounts of β-carotene. It was also reported that flowering of paprika increases as the temperature increases. So being a warm season crop paprika gets high light intensity and high temperature and thus produces large amounts of β-carotene. Topography plays very important role in β-carotene biosynthesis in fruits and vegetables as paprika grows in the areas of high altitude that favors high production

of β-carotene in paprika. When the estimated amount of β-carotene in paprika was compared to the amount of beta carotene in carrots the results revealed that it is high in paprika (Murthy and Murthy, 1963). Genetic reasons of variations in β-carotene concentration in different vegetables might be due to the protein which interacts with the initiator and TATA box is known as the TATA-box binding protein (TBP). TBP recognizes not only the core promoter of protein genes, but also RNA promoters. In different vegetables TBP acts differently, in paprika it may favors the production of β-carotene. (Ben-Dore *et al*., 2005) The strength of gene promoter and binding of regulatory protein at specific region may favor the high production of β-carotene as compared to other vegetables (Manitatis, 1989). The synthesis of β-carotene in paprika is also attributed to its peculiar plant physiology as compared to other vegetables (Shanmugawelu, 1985).

Carrots: The data related to the extraction and detection of β-carotene in carrots has revealed that the amount calculated in the recent studies (Fig. 7) was 6400 µg/100 gm which is much higher as compared to amount 4950 µgm/100 gm detected by Singh (1979).

The calculated amount 1.98 µg/mL in carrot was plotted on standard curve of β-carotene (Fig. 8) which was found in the range of standard amount of β-carotene which could be extracted from fruits or vegetables as described by Rangana (1997).

Figure 7 showed the difference in extraction of β-carotene in the present investigation from the earlier studies. The most important reason is related to stage of harvesting which affects the amount of β-carotene produced. The color of vegetables and fruits begins to change from green to red at the breaker stage of ripening. This transition is caused by the degradation of chlorophyll and establishment of β-carotene (Wills *et al*., 1981). In addition as discussed earlier the environmental factors included soil nutrient profile, topography, climate may also effect on the amount of β-carotene produced. The results revealed that carrots have beta carotene less than corn and paprika and almost equal to pumpkin, it was reported that the regulatory regions that are enhancers, silencer (DNA), boundary elements/insulators and promoter genes work collectively to direct the level of transcription of a particular gene (Albert, 1998). In carrots these may have less strength as compared to paprika and corn and equal to pumpkin. Genes in different plants decide to produce different things in different quantities, Gene for β-carotene production is normally known as B. The difference in the levels of mRNA of B, between different vegetables, could possibly be determined by transcriptional regulation of the B gene, which affects the amount of β-carotene produced. The property of the B-encoded enzyme as lycopene β-cyclase fits the

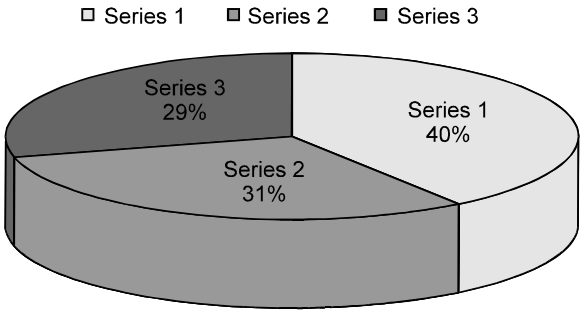

Fig.7: Comparative detection of β-carotene (µg/100 g) in carrots, (Series, 1) Amount calculated in this study, (Series 2) Amount Described by Singh (1979) and (Series 3) Amount described by Ranganna (1997)

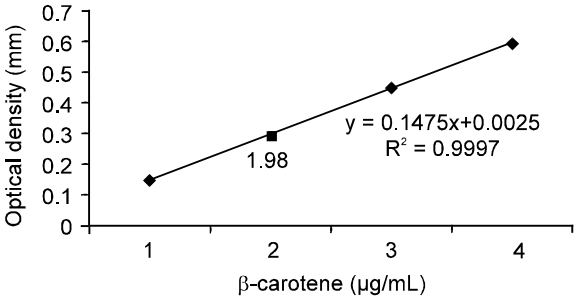

Fig. 8: Concentration of β-carotene in carrots (µg/mL)

phenotype of -carotene production in fruits of Beta, The detection of elevated mRNA level of B in Beta fruits explains the higher-carotene production (Chittaranjan, 2007). Some enzymes may also favor the production of β-carotene in greater amount in one vegetable and at the same time reduces its production in other vegetable. Carrots require large amounts of essential minerals; thus soil fertility must be high to obtain high yield and large nutrient contents High fertile soil may increase concentration of β-carotene in carrots (Shanmugawelu, 1985). Carrot is winter season crop so it receives low light intensity and low temperature as compared to corn and paprika and may be this is the reason of low β-carotene synthesis in carrots as compared to paprika and corn.

Pumpkin: Beta-carotene was extracted from pumpkin using organic solvent and then estimated by spectrophotometry as described in materials and methods. The data (Fig. 9) regarding the estimation of β-carotene from pumpkin revealed that the estimated quantity of β-carotene 3168 µg/100 gm was lower as compared to estimated 8735 µg/100 gm by Zang *et al*. (1989). The calculated amount 1.6 µg/mL in pumpkin was plotted on standard curve of β-carotene (Fig. 10).

The estimated concentration describes the accuracy of results as reported by Ranganna (1997) that β-carotene

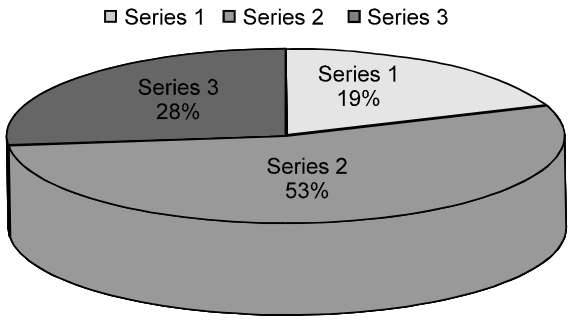

Fig. 9: Comparative detection of β-carotene (µg/100 g) in Pumpkin. (Series, 1) Amount calculated in this study, (Series, 2) Amount Described by Zang *et al*. (1989) and (Series, 3) Amount described by Ranganna (1997)

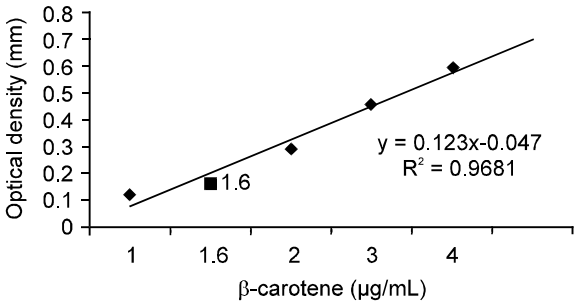

Fig. 10: Concentration of β-carotene (µg/mL) in pumpkin

concentration when extracted from fruit or vegetable should not exceed from 3 µg/mL. The low estimated β-carotene in pumpkin as compared to amount described by Zang *et al*. (1989) might be due to the difference in variety, agronomic practices and geographical distribution. Pumpkin is a warm season crop and receives maximum amount of high light intensity which increases concentration of β-carotene in vegetables but in this case it is apparent that possible reasons may be due to crop agronomic requirement, as it grows best in slightly acidic soils (Shanmugawelu, 1985) Acidic soils do not favor the high concentration of β-carotene in vegetables and fruits. This could be the reason for low β-carotene concentration in pumpkin. In addition the pumpkin has high phosphorous requirements than the nitrogen (Murthy and Murthy, 1963). So it had less amount of β-carotene as compared to other three vegetables and thus serves as a strong reason of low synthesis of β-carotene in pumpkin. Another possible reason might be that pumpkin attains the best growth and record good performance in tropical regions but β-carotene synthesis is favored in sub tropical regions that results in reduction of β-carotene as reported by Shanmugawelu (1985). The genetic variations may also bring variations in the concentration of β-carotene produced by the plant. The promoter is recognized by RNA polymerase, which then initiates transcription. In RNA synthesis, promoters are a means to demarcate which genes should be used for messenger RNA creation and, by extension, control which product the cell could manufacture and what will be its quantity (Chittaranjan, 2007). Hormones that can bind to the regulatory region of each gene enhance gene's action. The glucocorticoid receptor completes the combination of gene regulatory proteins required for efficient initiation of transcription and the genes are now switched on as a set. Pumpkin could produce β-carotene because genes for β-carotene production are switched on but quantity is low because the factors that enhance the action of gene to produce β-carotene are not very much active (Albert, 1998).

Cost evaluation: Cost of extraction of β-carotene was calculated by adding the costs of raw materials, electricity charges, solvent lost, unforeseeable expenses etc. (Table. 2) and were compared with the cost of synthetic β-carotene available in the market which was around Rs. 18000/kg.

It is evident from the table that the extracted cost of β-carotene from different vegetable sources were found much lower than the market cost. The local production will certainly lead to competition among producers for developing improved and economic production techniques resulting in reduced cost of production to save precious foreign exchange.

Conclusions: The indigenous production will certainly lead to competition among producers for developing improved and economic production techniques for β-carotene resulting in reduced cost of its production. It is evident from the results of the recent study that the soil and climatic conditions of Pakistan favors the substantial amount of beta carotene accumulation in most of the vegetables and we have large amounts of β-carotene in our fruits and vegetables as compared to most of the other regions in the world. The systematic extraction of this valuable compound will replace the use of synthetic coloring in food processing and also improve their functional potential. Further studies should be conducted to identify the concentration of β-carotene contents in other indigenous fruits and vegetables along with their environmental, genetic and seasonal variations.

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