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Determination of Water Soluble Vitamin in Fruits and Vegetables Marketed in Sindh Pakistan

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Abstract: Fresh vegetables and fruits are considered as a healthy source of water soluble vitamins. However water soluble vitamins may lose during washing and improper storage. Environmental factors such as light and temperature also have an effect on the moisture and vitamin contents of vegetables and fruits. Vitamins are needed for normal metabolic reactions in an appropriate amount, their deficiency or increased intake can lead to different irregularities in normal cellular metabolism. Pakistan is an agrarian country with about 21.9 million hectors of cultivation, of which Sindh province offers about 28554 acres .The production of vegetables and fruits is more than 50, 300 tons annually. The consumption of fruits and vegetables is increasing on daily basis as the people are getting more aware about the nutritional importance of fruits and vegetables. Vegetables and fruits supplies from the fields of harvesting to the markets are continuously in the exposure of environmental factors for example. Light intensity, traffic pollution which directly influence the moisture contents and ultimately reduces the water soluble vitamins contents. The vitamin contents also depend on the rate of the maturity of the vegetables and fruits as the early reaping is a common practice of the local farmers to get more economical benefits. Vegetables and fruits nutrients determination on monthly basis is a common practice in developed countries but such practices are rare in developing countries like Pakistan. Therefore the objective of the current study was the determination of vitamin B complex (B1, B2, B3, B6, B9) from the selected vegetables and fruits consume frequently in the region of Sindh, Pakistan. The HPLC analysis of acid hydrolysis samples showed that thiamin (B1) was found in the range of 0.02-0.18mg/100gm, riboflavin (B2) 0.016-0.2mg/100gm, niacin (B3) 0.01-0.1mg/100gm while pyridoxine (B6) was determined as 0.06-0.28mg/100gm and folate (B9) 0.016-0.19mg/100gm which have been determined lower than the recommended ranges.

Key words: Fresh vegetables, Vitamins, HPLC, Pakistan

INTRODUCTION

Vitamins are one of the indispensable and vital organic compounds provide no calorie but are important for cellular metabolic reactions. Vitamins are required in small and appropriate amount for normal growth and regulations (Verhoef *et al*., 1996). Deficit intake of the vitamins leads to the clinical malnutrition and continuous deficiency finally leads to malfunctioning of different enzymes (Vitale, 1976). Some of the vitamin cannot be produced in our cells from metabolic intermediates so are needed continuously for cellular enzymatic regulations (McCormick, 1996).

According to the solubility, vitamins categorized as fat soluble Vitamin e.g., A, D, E, K, where as water soluble Vitamins are Vitamin C and B complex comprised of thiamin, riboflavin, Niacin, pyridoxine and folate. Fat soluble vitamin A, D, E, K are able to store in our cells but excess amount of water soluble vitamin are excreted and cannot be absorbed. Vitamins are not only vital for the normal growth and regulation but also overcome different chronic diseases. According to Harvard studies addition of even one serving of fruits and vegetables have shown about 4% real improvement in cardiovascular symptoms (Hussein *et al*., 1999).

Fruits and vegetables contents of vitamins vary with small variations worldwide because of different environmental factors on soil, air and water as an Egyptian study on vitamin B1 in 240 varieties of foods have shown Thiamin range from 0.001-0.672mg/100g, while the recommended daily intake is 1.0mg (Bognar *et al*., 1998). Another study of Thiamin in food items done in Finland revealed the range of vitamin from 0.28-0.54 mg/100g (Food and Nutrition Board Rport, 2000). It has been reported that vitamin B1 (thiamin) is lost in food due to leaching and blenching on increased temperature (Serafini *et al*., 2002). According to a German study fruits and vegetables looses 31% of thiamin due to over cooking about 9% loose is by micro wave treatment (Aurea *et al*., 1998; Hertog *et al* ., 1993). Therefore analysis of vitamins is mandatory to maintain

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Table 1: Mean mg/100gm

Vitamins	CARROT (*Daucus carota*)	BRINJAL (*Solanum melongena*)	OKRA (*Hibiscus esculentum*)	SPINACH (*Spinaciaoleracea*)	BANANA (*Musa paradisiacal*)	GUAVA (*Psidium Guajava*)
B1, Thiamine	0.02	0.06	0.14	0.08	0.18	0.06
B2, Riboflavin	0.02	0.2	0.02	0.1	0.024	0.016
B3, Niacin	0.016	0.08	0.1	0.01	0.014	0.1
B6, Pyridoxine	0.06	0.18	0.28	0.26	--	0.16
B9, Folate	0.19	0.16	0.1	0.12	0.268	0.016

the vitamin sources data base (Cooksey *et al*., 1990). Its required intake should be compensated by eating other food sources as fresh legumes and grains (STRONG, 1947). The recommended thiamin intake for an adult is 1.1-1.2mg/day. A Brazilian study of Riboflavin determination with HPLC after enzymatic treatment have determine the Riboflavin contents in range 0.04-0.3 mg/100g (Toma *et al*., 1979). Niacin (B3) has two common types, available and unavailable or the total free Niacin. Methodology in HPLC for available Niacin demands the acid hydrolysis while non available Niacin is done with alkaline medium. Vitamin B6 is usually found in two types; pyridoxol and pyridoxamine. Pyridoxin is an essential vitamin and according to (FAO/WHO, 2002) the daily recommended allowances are 1.3mg/day. Green leafy vegetables are reported as the richest sources of folic acid (B9) but recent publication suggested that fruits and vegetables contain lower quantity of folic acid. (National Academy Press, Washington DC, 1996) A folic acid study done on lyophilized mixed vegetables with HPLC with microbiological extraction showed the folic acid range from 172-256µg/100g (Roughead *et al*., 1991). While according to the US recommendations of the folate dietary daily intake should be 400µg/day (Anderson *et al*., 1985).

Pakistan is an agrarian country with an area of 0.995 million hector which gives around 10.992 million tones production of fruits and vegetables annually (Raja *et al*., 1992). The nutrients of fruits and vegetables starts decreasing right from reaping because of improper storage and handling which leads to about 25-40% loss in vitamins. Studies on post harvest losses of nutrients in fruits and vegetables are rare, especially data on water soluble vitamin analysis in vegetable and fruits in Sindh is deficit.

MATERIALS AND METHODS

Sample collection: Vegetables (carrot, Brinjal, okra and spinach) and fruits (banana, guava) were purchased from different local retail Markets of Sindh, Pakistan. Fifteen samples of each fruit and vegetable was analyzed for vitamin analysis.

Sample preparation: All the vegetables and fruits were washed and dried, weighed 50 gm and cut into small pieces and extracted with 0.1 NHCL on water bath at suitable temperature and time period. All extracts were filtered through 0.40 micron filter and taken into 100 ml volumetric flask and volume was add up for mobile phase.

Standard preparation: Stock of standard (Sigma Aldrich Analytical grade Reagent) prepared by dissolving 0.01g of each standard in 100 ml of mobile phase followed by successive dilutions.

HPLC analysis: HPLC (Shimadzu, Model Prominence 20 A) equipped with UV detector and Supelco Discovery C-18 column (25 cm in length and 0.45 internal diameter) was used for analysis. Mobile phase was 50 m MK_2HPO_4 and MeOH (70:30) at 1 ml/min flow rate and 10µL of each sample/standard was injected and monitored at UV 254 nm.

RESULTS

Vitamin B1 (thiamin) determination was found higher in banana 0.018mg/100gm while other results are 0.06, 0.014, 0.006, 0.08mg/100gm in guava, okra, brinjal and spinach respectively as shown in (Table 1). Carrot had the minimum amount of thiamin 0.02mg/100gm. The reported daily intake is 1.0-1.2mg/day as by the study Alexander *et al*. (1984) B2 (riboflavin) had higher contents in brinjal 0.2mg/100gm and lower content in guava 0.016mg/100gm, B3 (Niacin) was found higher in okra and guava minimum contents in spinach. Daily recommended limit is 1.1-1.3mg/day. Vitamin B (6) was analyzed in carrot, brinjal, okra, spinach and guava 0.06, 0.18, 0.28, 0.26, 0.16mg/100gm, respectively, the recommended daily intake is 1.2-1.3mg/day. (McCormick, 1989) Banana shown 0.0268mg/100gm in B9 (niacin) which is maximum as compare to other analysis while guava showed 0.016mg/100gm the minimum amount. The daily permissible range for adults is up to 1000µg/day (Alexander *et al*., 1984).

DISCUSSION

Vitamins are one of the indispensable organic components of vegetables and fruits nutrients. In this study we have selected only water soluble B complex (B1, B2, B3, B6, B9) vitamins which are considered necessary for cellular metabolism especially carbohydrates metabolism. Vitamin analysis was done by HPLC methodology and only selected fruits and vegetables were taken for vitamin essay. It has been reported that more than 60% lose in vitamins have been

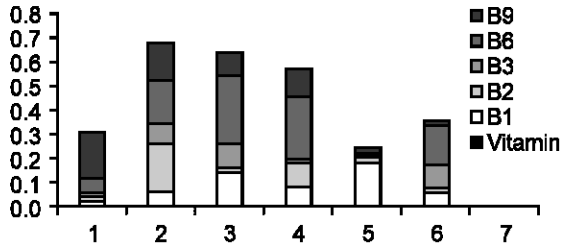

Fig. 1: Mean mg/100gm (B1, B2, B3, B6, B9) from (1) Carrot (2) Brinjal (3) Okra (4) Spinach (5) Banana (6) Guava

seen due to storage of vegetables and fruits. Vitamin content also depends on the rate of maturity of fruits and vegetables, as to reap the crop before maturity is a common practice of farmers to get economical benefits, while some studies have also suggested the HPLC methods less compatible for vitamin finding than other essays (Toma *et al*., 1979) so comparison of vitamin determination methods is recommended.

Conclusion: Current study was conducted on water soluble vitamins, thiamin, riboflavin, niacin, pyridoxine and folate from vegetables and fruits. The results has shown lower amounts than recommended limits, so food groups other than vegetables and fruits must be considered for balanced diet. There are two reasons which can affect vitamin determination values first the low safety measures in the market second; the selection of appropriate methodology for analysis. It is also recommended that the water soluble vitamin assay should be done as early as possible because high temperature and higher light intensity can loss the vitamin contents.

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