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Short Communication

Exploration of Locally Grown Yellow and Green Pumpkin as a Potential Source of β-Carotene and Vitamin A

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Abstract. Pumpkin is rich in beta-carotene which is used for preventing vitamin A deficiency. About 50 of carotenoids are the "Provitamin A compounds" which are precursors of retinol. Retinol is the active form of vitamin A. It plays an important role in vision. Beta carotene is an important anti-oxidant which protects the damaging of body cells by high free radical content because of its radical scavenging activity. The purpose of this study was to quantify the beta carotene and vitamin A present in yellow and green pumpkins. High performance liquid chromatography (HPLC) was used to analyze beta carotene and vitamin A. Yellow pumpkin contained the highest values of both the β-carotene and vitamin A than green pumpkin. The results illustrated that green pumpkin (*Curcurbita pepo*) possess 20.3µg/g beta carotene; 52.6µg/g vitamin A and yellow pumpkin (*Curcurbita maxima*) contains 35.7µg/g beta carotene and 60.2 µg/g vitamin A in their pulp. It was concluded that there is a need to promote the use of locally available sources of carotene and retinol to overcome nutrition deficiency in the people.

Keywords: pumpkin, provitamin A, retinol, HPLC, beta-carotene

Pumpkin is very effective for human health because of its carotenoids. Lutein, zeaxanthin, β–cryptoxanthin and β–carotene are the carotenoids present in pumpkin (Norshazila *et al.* 2014).

Pumpkin seeds have anti-diabetic effect. It lowers the elevated blood pressure and normalizes the electrocardiogram fluctuations (Patel, 2013). Beta-carotene is the originator of vitamin A. Pumpkin is rich in beta-carotene which is used for preventing vitamin A deficiency Seo *et al.* (2005). Carotenoids are categorized as carotenes which come from Latin word "carrots" (Karnjanawipagul *et al.* 2010). Synthetic beta carotene is also used as pills and lung cancer. Beta carotene can also be used as food additive. It is fat-soluble and can be added to food products study by Sahabi *et al.* (2012). The ability of capturing free oxygen and blue light in the retina, the xanthophylls lutein and zeaxanthin help to protect eyes against mascular degeneration connected with aging Kreck *et al.* (2006). It could be suggested that pumpkin, (*Cucurbita pepo*) pulp is a good supplement of protein, carbohydrate and fat with low anti nutrient (Adebayo *et al.* 2013). It is very necessary to adopt different better food strategies which allow optimal retention of vitamin A in order to overcome malnutrition of vitamin A (Djuikwo *et al.* 2011).

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The main purpose of this study was to identify the quantities of beta carotene and vitamin A present in yellow and green pumpkin varieties.

Samples preparation. The samples of yellow and green pumpkin were purchased from the local moon market of Lahore, Pakistan. Samples were prepared by peeling and chopping the fruits flesh into small pieces. The flesh of the pumpkin was then freeze-dried and saved for analysis.

Beta carotene extraction. 1gram of sample was taken in beaker and adds 0.1 g of $MgCO_3$. 25 mL of cold acetone was used to grind the sample mixture. The extract was then filtered15 mL of hexane and 10 mL of acetone extract was taken in a separating funnel and left the mixture for 20 min. 50 mL of distilled water was added for washing and lower aqueous phase was discarded. The sample washing with water was repeated

twice. The upper organic layer was transferred to 25 mL measuring flask having 4.5 mL acetone. Volume was made with hexane.

Preparation of standard solutions for beta carotene analysis. To prepare stock solution of beta carotene, standard of 0.01g was dissolved in 10 mL hexane and volume was made up to 100 mL with hexane. From stock solution, 20 µg / mL was prepared. Different concentrations of 1, 2, 3, 4, 5, 6 and 7 µg / mL standard solutions were prepared. The readings were obtained by using HPLC (High Performance Liquid Chromatography) system.

Detection of vitamin A. 10g sample was taken in a beaker and 40 mL ethanol and 10mL of 50% KOH were added and agitated the mixture. Then 0.3g ascorbic acid was added in the mixture and heat on water bath at 80 °C for 30 min.The mixture was then poured into the separating funnel and 50mL (1:1) hexane: ether was added four times, for the extraction of vitamin A in ether layer. Then the concentrations of vitamin A in both yellow and green pumpkin were determined by using HPLC system.

Chromatographic analysis. The incidence of beta carotene and vitamin A in collected samples were analyzed by using HPLC equipped with a Lichrospher 100 column reverse phase 18 of Merck with 5µm, 250 mm long x 4 mm internal diameter, high-pressure pump, manual injector with a 50-µ L size loop and UV-visible spectrophotometer detector (model SPD 10 AV, Shimadzu). Nitrogen flow was used to evaporate 2 mL of the extract stored in petroleum ether and re-suspended in acetone. 20 to 30µL filtered extracts from 0.45-µm filter unit were injected into the chromatographic column (Pinheiro-Sant'Ana *et al*. 1998). The mobile phase used was methanol: acetonitrile: ethyl acetate (80:10:10). 2.0 mL/min was the flow rate of the mobile phase and run time was 9-10 min for beta carotene and 7 min. for vitamin A. The β -carotene eluted was detected and quantified using a UV-visible detector attached to the HPLC system. The retention time (Rt) and peak areas of appropriate standard (β-carotene and vitamin A) were used to identify and quantify the isolated β-carotene and vitamin A.

Determination of β-carotene and vitamin A was made according to the formula.

$$C(\mu g/g) = \frac{Ax \times Cs \left(\mu \frac{g}{mL}\right) \times V\,(mL)}{As \times P\,(g)}$$

where:

Ax = Carotenoid peak area; Cs = Standard concentration; As=Standard area; V=Total extract volume and P=Sample weight. The wavelength of UV/visible detector used was 450 nm for β-carotene and vitamin A.

In Table 1, the β- carotene content of green and yellow pumpkins can be observed. The results illustrated that green pumpkin (*Curcurbita pepo*) possess 20.3µg/g beta carotene and yellow pumpkin (*Curcurbita maxima*) contains 35.7 µg/g beta carotene in their pulp.

In Table 1, the vitamin A content of green and yellow pumpkins can be observed. The results illustrated that green pumpkin possess 52.6 µg/g vitamin A and yellow pumpkin contains 60.2µg/g vitamin A in their pulp. The content of beta carotene in yellow pumpkin was more than green pumpkin. The comparison between the peak areas (absorbance) of green and yellow pumpkins with regard to standard of vitamin A is described in Table 2. This indicates that yellow pumpkin have more level of vitamin A than green pumpkin.

Figure 1. Elaborated the fact that green pumpkin has lower absorbance value 0.82mL volts than Yellow pumpkin which has 0.65 millivolts absorbance in the retention time of 9-10 min.

Figure 2 shows that green pumpkin has lower absorbance value 0.04 mLvolts than yellow pumpkin which has 0.07 milli volts absorbance in the retention time of 7 min. Sahabi *et al.* (2012) reported that yellow pumpkin (*Curcurbita maxima*) contains 38.6 µg/g of beta carotene that is in approximation with the present study. According to Sungpuag *et al*. (1999), β-carotene of hand peeled pumpkin is 176 µg/100g which is higher than this endeavor.

The results of the present study are nearly similar to Kurz *et al.* (2008) that is 20.3 µg/g beta carotene in pumpkin. It was illustrated that a harmony observed for all cultivars was the presence of relatively high contents of β-carotene, ranging from 4.73 µg/g to 27.14

Table 1. Quantitative analysis of beta-carotene and vitamin A

Samples	Beta-carotene (µg/g)	Vitamin A (µg/g)
Green pumpkin	20.3	52.6
Yellow pumpkin	35.7	60.2

Table 2. Comparison between green and yellow pumpkins for beta-carotene & vitamin A analysis

Samples	Beta-Carotene		Vitamin A	
	Absorbance (mV)	Retention time	Absorbance (mV)	Retention time
Standard solution	0.99	9-10 min	0.09	7 min
Green pumpkin	0.65	9-10 min	0.04	7 min
Yellow pumpkin	0.82	9-10 min	0.07	7 min

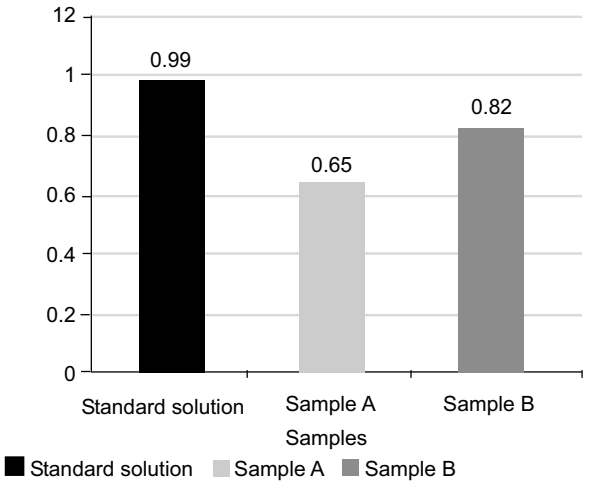

Fig. 1. Difference in absorbance of the samples for beta-carotene (sample A = green pumpkin; sample B = yellow pumpkin).

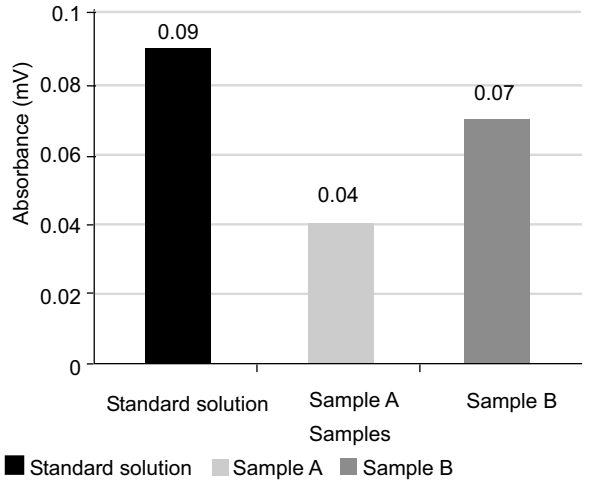

Fig. 2. Difference in absorbance of the samples for vitamin A (sample A = green pumpkin; sample B = yellow pumpkin).

µg/g. Horbowicz *et al.* (2004) have studied that β-carotene content in fruit flesh of pumpkin ranged from 0.07 to 6.07 mg/100g for whole (Norshazila *et al.* 2014). The carotenoids detected were in range of 29.16 mg/100g to 154.76 mg/100g. It was concluded that the vitamin A from 1g of dry weight pumpkin may between 49.65µg to 260.66µg. These are in similar range of vitamin A in pumpkin derived in the current study which is 52.6 µg/g-60.2 µg/g. In a similar study conducted by Liu *et al*. (2016), the comparison of 8 different pumpkin varieties was made for β-carotene contents by HPLC method. The β-carotene contents were found in eight varieties of pumpkin fruit ranged between 2.29-4.22 mg/100 g fresh weight. The results showed that there was a significant difference between beta carotene in different varieties of pumpkins.

Results of the study are also coinciding with the finding of Itle and Kabelka (2009) who stated that β-carotene in summer type squash (*C. pepo*) were 0.0 to 21.3µg/g, 0.3 to 1.7 µg/g and 0.6 to 23.0 µg/g, respectively. In winter-type squash (*C. moschata*and *C. maxima*), β-carotene varies from 7.1 to 74.0 µg/g for whole. Kim *et al.* (2012) suggested that nutrient compositions may differ significantly among different pumpkin parts and species. The β-carotene contents of the flesh in *C. pepo* was (1.48mg/Kg raw weight) and *C. Moschata* (5.70 mg/Kg raw weight) were lower than *C. maxima* (17.04 mg/Kg raw weight). In the present study, it is described that beta carotene is more in Yellow pumpkin (*Cucurbita maxima*) than Green pumpkin (*Cucurbitapepo*) as Kim *et al.* (2012) suggested. Khoo *et al*. (2011) have studied that *Cucurbita maxima* pumpkin contains 0.06-14.85 mg/100g β-carotene (Saini *et al*., 2015). The antioxidant perspective of beta carotenoids is of great importance to human health and a carotenoids rich diet is suggested for the deterrence of different age-related and chronic diseases (Raikos, 2017).

From the present study it becomes evident that the beta-carotene and vitamin A contents are higher in Yellow pumpkin than that of Green pumpkin. The results showed that consumption of pumpkin fruits can enhance the amounts of vitamins A and beta-carotene consumed.

Conflict of Interest. The authors declare no conflict of interest.

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