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Techniques for Determination of Vitamin B_6, Vitamin C and Variability in Areca Nut (*Areca catechu*) Samples of Karnataka, India

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A B S T R A C T

Vitamin B6, also called pyridoxine, is one of the B vitamins. It helps the body to convert food (carbohydrates) into fuel (glucose), which is used to produce energy. A simple and sensitive reversed-phase (RP) HPLC method was used for determination of vitamin B6 present in areca nut samples. Areca nut samples were collected from Shimoga, Davanagere, Chikkamagalur, Chitradurga, Dakshina Kannada and Udupi districts of Karnataka, India. RP- HPLC analysis was performed with UHPLC system (Thermo fisher-ultimate 3000) equipped with UV/Visible detector and C_{18} column. The column effluents were monitored at 220nm. Vitamin B6 in different district ranged from 10 to 91 ppm. The mean vitamin B6 content was lowest in Shimoga district (15.68 ppm) and highest in Dakshina Kannada district (50.49 ppm). Vitamin C is a most important vitamin in the human diet, excessively present in fruits and vegetables. It is also known as ascorbic acid and is water-soluble. In areca nut vitamin C is present in trace amount. The dried powdered areca nut samples were used for the estimation of vitamin C content and it was determined by redox titration with potassium iodate in the presence of potassium iodide. The titration method is very simple and fast method. The Concentration of vitamin C varied in areca nut samples collected from different district of Karnataka. Highly significant variations existed in Vitamin C content among samples collected from 35 taluks of 6 districts. The range of Vitamin C was 264-1408 ppm among 117 samples collected from hoblies of Karnataka. The variation may be due to varietal differences; mode of cultivation, soil types and processing methods.

Introduction

Vitamins are an extremely diverse range of organic compounds present in minute amounts in natural foodstuffs. They are vital in the enzyme reactions that are necessary for carbohydrate, fat, and protein metabolism. Vitamins are classified into two groups: water-soluble and fat-soluble. Vitamins are relatively unstable, affected by factors such as heat, light, air, other food components and even food processing conditions. Vitamin play a critical role in nutrition. Food manufacturers face a challenging task due to their relative instability and it is important to have their qualitative and quantitative analysis for nutrition and food industry.

Vitamin C also known as ascorbic acid is a water soluble vitamin. Except for human and

other primates, most of the phylogenetically higher animals can synthesize vitamin C. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables.

Vitamin C naturally present in oranges, grapefruits, lemons, limes, green and red peppers, tomatoes, strawberries, spinach, mangos, winter squash, cantaloupe, broccolli, potatoes and areca nuts. In areca nut, vitamin C is present in trace amount. Vitamin C is needed for proper growth, development and also to heal wounds. It is used to make the collagen tissue for healthy teeth, gums, blood vessels and bones. Since, vitamin C is a powerful antioxidant, it is also used to prevent damage to our bodies from toxic substances and pollutants, such as cigarette smoke. Vitamin C is an important part of our nutrition and is also an antioxidant. A lack of vitamin C in our bodies causes scurvy, a disease that causes teeth and bone abnormalities (Vasanth kumar *et al.*, 2013). Several methods are available for measurement of ascorbic acid such as HPLC, spectrophotometric and titration, enzymatic methods for the analysis of vitamin C. The vitamin C concentration in areca nut was determined by titration method. The titration method is very simple and quick. This method determines the vitamin C concentration in areca nut with potassium iodate (KIO_3) in the presence of potassium iodide (KI).

Vitamin C is determined directly with iodine and iodate solution in a redox titration, using starch as indicator, which reacts rapidly and stoichiometric with iodine to give iodide ions, while it is oxidized to dehydroascorbic acid. Once all the ascorbic acid is oxidized, the excess iodine solution will react with the starch indicator, forming a blue-dark starch-iodine complex as end point of titration (Vitor *et al.*, 2013). This method is suitable for the determination of vitamin C content in fruits, vegetables and nuts.

Materials and Methods

Determination of vitamin B6 by the RP-HPLC method

Areca nut samples were collected from different districts of Karnataka (Shimoga, Davangere, Chikkamagalur, Chitradurga, Udupi, and Dakshina kannada). Vitamin B6 was analysed using a Discovery C18 reversed-phase HPLC column, 15cm x 4.6mm ID, 5µm particles.

Since each vitamin has its own maximum absorbance at a different wavelength, we used 220nm as a compromise to detect vitamins in the sample. In the mobile phase, we attempted to use simple buffers without adding ion pairing agents or competing for amine modifiers. Water soluble vitamins are very hydrophilic.

They can be eluted from reversed-phase columns with low concentrations of aqueous methanol or acetonitrile. Water-soluble vitamin pyridoxine standard was analyzed by reversed-phase HPLC on a Discovery C18 column.

Estimation of vitamin C by titrimetric method

The iodometric titration method was used to estimate the amount of vitamin C in areca nut samples. The samples were powdered. 2g of dried powdered areca nut samples were taken and kept in a 250ml of conical flask and added with 50ml of distilled water. Further, 6 ml of 1N hydrochloric acid, 0.25 g of KI and 1ml of starch indicator solution were added.

Titrate the sample with the 0.01 N potassium iodate solution. The end point of the titration was dark blue color complex. Repeated the titration with aliquots sample solution until concordant values were obtained.

Results and Discussion

Vitamin B6 in areca nut

The results of Vitamin B-6 in areca samples of Karnataka have been presented in table 1 and 2. In Shimoga district high amount of vitamin B6 was estimated in Anwatti and Kasaba hoblies (52.33 and 47.67 ppm) of Soraba taluk and 10 ppm of vitamin B6 was determined in different hoblies of Shimoga district. In Chikkamagalur district also 10 ppm of vitamin B6 was determined in different hoblies. The high amount of vitamin B6 was determined in Kigga hobli (72.67 ppm) of Koppa taluk followed by Sakharayapattana hobli of Kadur taluk (69.00 ppm). High amount of Vitamin B6 was estimated in Bilichodu hobli (84.67 ppm) followed by Santhebennuru hobli (55.00 ppm) of Davanagere district. High content i.e., 72.67 and 68.33 ppm was estimated in Turuvanooru and Bharamasagar hoblies of Chitradurga district. In Dakshina Kannada district highest amount of vitamin B6 was determined in Upinangadi (91.33 ppm) and Surathkal (91.00 ppm) hoblies of Puttur and Mangalore taluks respectively. In Udupi district high amount of vitamin B6 was determined in Ajekaru hobli (81 ppm) followed by Karkala hobli (56 ppm) of Udupi district (Table 1).

In Shimoga high amount of vitamin B6 was determined in Soraba taluk (23.80 ppm) followed by Shimoga taluk (18.21 ppm) and less content was determined in Hosanagara taluk (Table 2). In Sringeri taluk of Chikkamagalur district significantly high content was estimated compare to other taluks. There was a significant difference in vitamin B6 content was estimated in different taluks of Davanagere district. In Dakshina kannada district less content of vitamin B6 was determined in Sullia taluk (33.00 ppm) compared to other taluks. In Udupi district high amount of vitamin B6 was estimated in Karkala taluk (67.98 ppm) followed by Brahmavara taluk (32.53 ppm) of Udupi district.

Vitamin C in areca nut

The amount of Vitamin C present in different areca nut samples is tabulated in table 3 and 4. Titration method was used to determine the vitamin C concentration. In Shimoga district there was no significant difference of vitamin C content. The higher concentration of vitamin C was observed in Shikaripura taluk (1139.20 ppm) and followed by Sagara (985.76ppm), Hosanagara (968.91ppm) respectively. In Shimoga and Sorab vitamin C content was 704.13 and 587.97ppm.

In Chikkamagalur district vitamin C content was more in Sringeri (1188.96ppm), followed by Tarikere (1056.31ppm) and Chikkamagalur (1003.12ppm) and less amount was identified in NR pura taluk (661.16ppm).

In Davanagere there was no much variation in vitamin C content. There was variation between 924.94ppm to 1145.57ppm in different taluks of Davangere district.

In Chitradurga district higher concentration was determined in Hosadurga and Holalkere (528.25 and 528.13ppm) and less concentration was found in Chitradurga and Hiriyur (462.77ppm and 396.24ppm).

There was a significant difference of vitamin C content in Dakshina Kannada district. Higher concentration was estimated in Kadaba 794.16ppm and followed by Bantwala and Sullia (704.83ppm and 661.62ppm), and less amount of vitamin C concentration was found in Mudabidre taluk (265.52ppm). In Udupi district vitamin C content was more in Bramhavara taluk (925.13ppm). The less amount of vitamin C content was found in Baindur and karkalla (265.73 and 265.19ppm).

Table.1 Estimation of vitamin B-6 (ppm) in areca nut collected from different locations of Karnataka

Districts	Shimoga	Chikkamagalur	Davanagere	Chitradurga	Dakshina Kannada	Udupi
locations						
1.	17.67 (Nidige)	10.00 (Ambale)	51.67 (Ubrani)	10.00 (Talya)	10.00 (Panemangalore)	54.00 (Kota)
2.	13.00(Aynur)	10.00 (Alduru)	25.00 (Kasaba)	72.67 (Turuvanooru)	68.67 (Bantwala)	10.00 (Brahmavara)
3.	15.67 (Kumsi)	15.00 (Avathi)	21.00 (Basavapattana)	10.00 (Talaku)	40.67 (Vitla)	10.00 (Bainduru)
4.	10.00 (Haranahalli)	13.67 (Kasaba)	10.00 (Anagodu)	68.33 (Bharamasagar)	37.00 (Kokkada)	81.00 (Ajekaru)
5.	12.00 (Holalur)	10.00 (Khandya)	55.00 (Santhebennur)	48.67 (Nayakanahalli)	45.00 (Belthangadi)	67.98 (Karkala)
6.	21.33 (Kasaba)	12.33 (Yagati)	51.33 (Kasaba)	43.33(Parashurampura)	40.33 (Venuru)	23.67 (Kundapura)
7.	10.00 (Kudlugere)	40.33 (Hirenalluru)	40.00 (Mayagonda)	33.67 (Ramagiri)	81.00 (Kadaba)	23.67 (Vandse)
8.	37.67(Holehonnur)	35.33 (Panchanahalli)	10.00 (Arasikere)	50.67 (Hiregunturu)	10.00(Gurupura)	10.00 (Udupi)
9.	24.33 (Talaguppa)	10.00 (Birur)	23.67 (Kasaba)	32.33 (Imangala)	10.00(Malki)	45.00 (Kapu)
10.	10.00 (Banagi)	16.33 (Chaulahiriyur)	40.33 (Chigateri)	34.67 (Kasaba)	82.33 (Mudabidre)	-
11.	10.00 (Anandapura)	49.67 (Singatagere)	14.00 (Telagi)	40.00 (J.G.Halli)	91.00 (Surathkal)	-
12.	15.33 (Aninahalli)	10.00 (Kasaba)	42.67 (Kasaba)	-	55.33 (Kasaba)	-
13.	10.00 (Karooru)	69.00 (Sakharayapattana)	51.33 (Malebennuru)	-	91.22(Uppinangadi)	-
14.	10.00 (Nagara)	50.00 (Megunda)	28.67 (Kasaba)	-	88.38.00 (Puttur)	-
15.	37.33 (Kerehalli)	15.33 (Hariharapura)	10.00 (Govinkovi)	-	56.00 (Sullia)	-
16.	10.00 (Huncha)	42.67 (Kasaba)	10.00 (Belagutti)	-	-	-
17.	10.00 (Agumbe)	42.67 (Kalasa)	10.00 (Saswehalli)	-	-	-
18.	11.33 (Muthuru)	10.00 (Kasaba)	16.00 (Kasaba)	-	-	-
19.	10.00 (Mandagadde)	72.67 (Kigga)	84.67 (Bilichodu)	-	-	-
20.	10.00 (Agrahara)	10.00 (Gonibidu)	-	-	-	-
21.	10.00 (Kuppagadde)	10.00 (Banakal)	-	-	-	-
22.	10.00 (Uluri)	13.00 (Balooru)	-	-	-	-
23.	13.67 (Chandragutti)	10.00 (Kasaba)	-	-	-	-
24.	12.00 (Jade)	11.33 (Balehonnuru)	-	-	-	-
25.	12.00 (Udugani)	10.00 (Ajjampura)	-	-	-	-
26.	13.67 (Talagunda)	10.00 (Amruthapura)	-	-	-	-
27.	47.67 (Kasaba)	-	-	-	-	-
28.	11.33 (Hosuru)	-	-	-	-	-
29.	10.00 (Anjanapura)	-	-	-	-	-
30.	52.33 (Anwatti)	-	-	-	-	-
31.	13.00 (Bhadravathi)	-	-	-	-	-
32.	10.00 (Anaveri)	-	-	-	-	-
33.	10.00 (kallihal)	-	-	-	-	-
34.	10.00 (shiralkoppa)	-	-	-	-	-
35.	10.00 (Kasaba)	-	-	-	-	-
36.	10.00 (Shikaripura)	-	-	-	-	-
Mean	41.33 (Kasaba)	23.43	31.33	42.39	53.62	34.81
P 0.01	3.49**	18.00**	9.10**	8.40**	8.71**	10.40**
Range	52.33-10.00	72.67-10.00	84.67-10.00	72.67-10.00	91.33-10.00	81.00-10.00
CV (%)	12.38	11.29	16.59	11.29	9.856	17.19
S.Em±	1.165	6.074	3.001	2.796	2.897	3.456

Note: Name of locations (hoblies) are indicated in parenthesis

Table.2 Assessment of vitamin B_6 (ppm) of Areca nut samples in different Taluks of Karnataka

Districts Taluks	Shimoga	Chikkamagalur	Davanagere	Chitradurga	Dakshina Kannada	Udupi
1.	18.21 (Shimoga)	11.52 (Chikkamagalur)	35.98 (Chennagiri)	40.61 (Chitradurga)	39.51(Bantwala)	32.53(Brahmavara)
2.	12.64 (Bhadravathi)	35.10 (Kadur)	24.09 (Davanagere)	44.41 (Hiriyur)	41.50 (Belthangadi)	10.24 (Baindur)
3.	15.71(Sagar)	33.53(Koppa)	27.21 3(Harihara)	35.52(Holalkere)	40.34(Kadaba)	67.98(Karkala)
4.	10.21 (Hosanagar)	42.04 (Sringeri)	25.40 (Honnali)	56.44 (Hosadurga)	55.08 (Mangaluru)	23.80 (Kundapura)
5.	12.77 (Thirthahalli)	11.40 (Mudigere)	51.04 (Jagalur)	-	55.67 (Mudabidre)	27.49 (Udupi)
6.	23.80(Sorab)	10.62(N R Pura)	-	-	88.38(Puttur)	-
7.	16.41 (Shikaripura)		-	-	33.00(Sullia)	-
Mean	15.68	24.04	32.74	44.24	50.49	32.40
P 0.01	0.69**	0.80**	0.88**	0.48**	0.60**	0.88**
CV	0.89	1.02	1.96	0.78	0.90	1.93
S.Em±	0.23	0.26	0.29	0.15	0.20	0.28

Note: Name of taluks are indicated in parenthesis

Table.3 Determination of Vitamin-C content (ppm) of areca nut samples from selected districts of Karnataka

Districts Hoblies	Shimoga	Chikkamagalur	Davanagere	Chitradurga	Dakshina Kannada	Udupi
1.	352.33 (Nidige)	1057.29 (Ambale)	792.65 (Ubrani)	527.95 (Talya)	528.46(Panemangalore)	792.22 (Kota)
2.	1056.28(Aynur)	1320.95 (Alduru)	1056.08 (Kasaba)	527.47 (Turuvanooru)	792.52 (Bantwala)	527.93(Brahmavara)
3.	704.97(Kumsi)	1056.45 (Avathi)	1321.04(Basavapattana)	528.64 (Talaku)	792.50 (Vitla)	264.55 (Ajekaru)
4.	704.36 (Haranahalli)	528.86 (Kasaba)	1056.33(Anagodu)	264.79(Bharamasagara)	528.50 (Kokkada)	264.68(Bainduru)
5.	705.16(Holalur)	528.68 (Khandya)	1056.33 (Santhebennur)	528.45 Nayakanahalli)	528.23(Belthangadi)	264.56(Karkala)
6.	352.11 (Kasaba)	1056.40 (Yagati)	1056.50 (Kasaba)	528.18 (Parashurampura)	528.71 (Venuru)	528.16(Kundapura)
7.	1053.55 (Kudlugere)	792.91 (Hirenalluru)	1056.53(Mayagonda)	264.56 (Ramagiri)	792.79 (Kadaba)	264.37 (Vadse)
8.	704.05 (Holehonnur)	528.22 (Panchanahalli)	1321.13 (Arasikere)	264.79 (Hiregunturu)	528.41 (Gurupura)	528.88(Udupi)
9.	1056.66 (Talaguppa)	1321.22 (Birur)	1056.28 (Kasaba)	264.75 (Imangala)	264.75 (Mangaluru)	528.48 (Kapu)
10.	1056.82 (Banagi)	792.80 (Chaulahiriyur)	1056.40 (Chigateri)	792.63(Kasaba)	264.56 (Mudabidre)	-
11.	704.27 (Anandapura)	528.96 (Singatagere)	792.90 (Telagi)	528.48 (J.G.Halli)	528.53 (Surathkal)	-
12.	1055.36 (Avinahalli)	545.67 (Kasaba)	1056.50 (Kasaba)		628.64 (Kasaba)	-
13.	756.33 (Karooru)	792.80 (Sakharayapattana)	792.51 (Malebennuru)		528.36(Uppinangadi)	-
14.	1049.34 (Nagara)	528.97 (Megunda)	792.51 (Kasaba)	-	528.75(Puttur)	-
15.	1056.66 (Kerehalli)	793.12 (Hariharapura)	1321.17 (Govinakovi)	-	528.52(Sullia)	-
16.	704.75 (Huncha)	1056.84 (Kasaba)	1056.74 (Belagutti)	-	-	-
17.	1056.81 (Agumbe)	1056.53 (Kalasa)	793.53 (Saswehalli)	-	-	-
18.	1056.81(Muthuru)	1056.51 (Kasaba)	793.11 (Kasaba)	-	-	-
19.	704.83 (Mandagadde)	1056.53 (Kigga)	1056.33 (Bilichodu)	-	-	-
20.	1056.77 (Agrahara)	732.33 (Gonibidu)	-	-	-	-
21.	1056.54 (Kuppagadde)	528.67 (N R Pura)	-	-	-	-
22.	1055.80 (Uluri)	792.35 (Balooru)	-	-	-	-
23.	704.33 (Chandragutti)	528.22 (Kasaba)	-	-	-	-
24.	1408.14 (Jade)	528.22 (Lakya)	-	-	-	-
25.	705.05 (Udugani)	1321.16 (Balehonnur)	-	-	-	-
26.	1053.55 (kasaba)	792.75 (Ajjampura)	-	-	-	-
27.	1056.77 (Kasaba)	-	-	-	-	-
28.	352.46 (Hosuru)	-	-	-	-	-
29.	704.59 (Anjanapura)	-	-	-	-	-
30.	1056.48 (Anwatti)	-	-	-	-	-
31.	352.67 (Bhadravathi)	-	-	-	-	-
32.	704.58 (Anaveri)	-	-	-	-	-
33.	1056.32 (Kallihal)	-	-	-	-	-
34.	1408.66 (Shiralkoppa)	-	-	-	-	-
35.	1056.77 (Kasaba)	-	-	-	-	-
36.	950.33 (Shikaripura)	-	-	-	-	-
37.	704.87 (Kasaba)					
Mean	873.9	812.89	6517.7	456.42	552.8	440.4
Range	352-1408	528-1321	792-1321	264-792	264-792	264-792
P 0.01	6.20**	11.28**	0.90**	0.62**	0.36**	0.48**
CV (%)	0.423504074	0.743854	0.049548	0.068675	0.035327	0.056452
S.Em±	2.07685281	3.72765	0.294337	0.185358	0.114538	0.143547

Note: Name of hoblies are indicated in parenthesis

Table.4 Vitamin - C content (ppm) of Areca nut samples from selected from districts of Karnataka

Districts / Taluks	Shimoga	Chikkamagalur	Davanagere	Chitradurga	Dakshina Kannada	Udupi
1.	704.13 (Shimoga)	1003.12 (Chikkamagalur)	1056.45 (Chennagiri)	462.77 (Chitradurga)	704.83 (Bantwala)	925.13 (Brahmavara)
2.	924.41 (Bhadravathi)	718.95 (Kadur)	1145.57 (Davanagere)	396.24 (Hiriyur)	527.92 (Belthangadi)	265.73 (Baindur)
3.	985.76 (Sagara)	704.55 (Koppa)	924.94 (Harihara)	528.13 (Holalkere)	794.16 (Kadaba)	265.19 (Karkala)
4.	968.91 (Hosanagar)	1188.96 (Sringeri)	1012.96 (Honnali)	528.25 (Hosadurga)	476.13 (Mangaluru)	395.84 (Kundapura)
5.	968.82 (Thirthahalli)	948.11 (Mudigere)	925.52 (Jagalur)	-	265.52 (Mudabidre)	528.59 (Udupi)
6.	587.97 (Sorab)	661.16 (N R Pura)	-	-	529.07 (Puttur)	-
7.	1139.20 (Shikaripura)	1056.31 (Tarikere)	-	-	661.62 (Sullia)	-
Mean	897.02	897.31	1012.99	478.85	565.61	476.07
P 0.01	1.12**	1.18**	1.29**	0.52**	0.42**	0.46**
CV (%)	0.0727	0.07194	0.074553	0.143462	0.112757	0.126059
S.Em±	0.376515	0.372693	0.436063	0.396618	0.368211	0.346506

Note: Name of taluks are indicated in parenthesis

Table.5 List of important ingredients used for wet processing of areca

Sl No	Shimoga		Chikamagalur		Davanagere		Chitradurga		D.K		Udupi	
	Ingredient	%	Ingredient	%	Ingredient	%	Ingredient	%	Ingredient	%	Ingredient	%
1	Jaggery	59	Jaggery	100	Jaggery	100	Kaachu	71	-Dry Processing-		-Dry Processing-	
2	Cooking oil	48	Jamun bark	86	Cooking oil	92	Jaggery	71				
3	Jamun tree bark	43	Betel leaf	52	Lime	92	Jamun tree bark	57				
4	Betel leaf	43	Cooking oil	72	Betel leaf	92	Lime	43				
5	Banana leaf tip	24	Lime	52	Jamun tree bark	64	Betel leaf	36				

Vitamin C content in different hoblies of different district of Karnataka is tabulated in table 2. Vitamin C content was significantly high in almost all the hoblies of Shimoga district. Banagi and Muturu hobli were having (1056.82 and 1056.81ppm) of vitamin C content and followed by kasaba hobli of Hosanagara taluk (1056.77ppm), Talaguppa hobli (1056.66ppm) and kasaba hobli of Bhadravathi taluk (1056.55ppm). Less concentration was identified in kasaba hobli of Shimoga taluk 352.11ppm, nidige hobli 352.33ppm respectively.

In Chikkamagalur district, more vitamin c content was found in Kasaba hobli of Sringeri taluk (1056.84ppm), Kalasa and Kasaba hobli of Mudigere taluk had 1056.53 and 1056.51ppm of vitamin C content and less concentration was estimated in Lakya and khandya hobli of Chikkamagalur taluk (528.22 and 528.68ppm), and Kasaba hobli of Narasimharajapura taluk (528.67ppm).

There was no significant difference of vitamin C content in Davangere district. The vitamin c ion concentration was similar in Santhebennuru and Anagodu hobli (1056.33ppm). In Kasaba hobli of Honnali taluk and in Belaguthi hobli vitamin C content was found to be (1056.50 and 1056.74ppm).

In Chitradurga district there was a significant variation of vitamin C content. The highest concentration of vitamin C was found in Kasaba hobli of Holalkere taluk (792.63ppm) and less content was observed in Ramagiri hobli (264.56ppm) and followed by Imangala hobli, Hireguntanuru hobli (264.75ppm and 264.79ppm).

In Kadaba hobli of Dakshina kannada district were having more concentration of vitamin C content (792.79ppm), and followed by Bantwala and vitla hobli (792.52 and 792.50ppm) and Mangaluru B and Mudabidre were having less content of vitamin C (264.75 and 264.56ppm). In Udupi district high concentration of vitamin C was identified in Kota hobli (792.22ppm) and less content was found in Vandse hobli 264.37ppm and followed by Ajekaru and Karkalla hobli of karakala taluk (264.55ppm and 264.56ppm).

Vitamin B_6, also called pyridoxine, is a water-soluble nutrient that is part of the B vitamin family. B vitamins, including vitamin B6, help support adrenal function, help calm and maintain a healthy nervous system, and are necessary for key metabolic processes.

Vitamin B6 acts as a coenzyme in the breakdown and utilisation of carbohydrates, fats and proteins. Vitamin B_6 helps in the production of neurotransmitters, the chemicals that allow brain and nerve cells to communicate with one another, ensuring that metabolic processes such as fat and protein metabolism run smoothly, and is important for the functioning of immune system in older individuals.

The result of samples analysed for vitamin B6 from 117 locations of six important areca growing districts of Karnataka are presented in the table 1. In general, the contents of vitamin B6 ranged from 10 to 91 ppm. Higher vitamin B6 contents were in Anwatti (52.33), Kigga (72.67), Bilichodu (84.67), Turuvanooru (72.67), Uppinangadi (91.22) and Ajekaru (81.00) of Shimoga, Chikkamagaluru, Davanagere, Chitradurga, Dakshina kannada and Udupi districts respectively. It is interesting to notice that, in about 30 percent of locations the content of vitamin B6 was only 10 ppm. Talukwise variation was highly significant with a range of 10.24 to 88.38 ppm. Highest vitamin B6 was observed in samples of Puttur (88.38) followed by Karkala (67.98) and Hosadurga (56.44) whereas lowest concentration was in Hosanagara (10.21). The mean vitamin content was lowest in Shimoga district (15.68) and highest was in Dakshina Kannada district (50.49).

Regarding vitamin C contents, there were significant differences among the hoblies of all the six districts of Karnataka. Vitamin content in different hoblies has been presented in table

3. The samples of 8 hoblies *viz.* Alduru, Panchanahalli, Gonibidu, Balehonnuru, Basavapatna, Kasaba, Arsikere and Govinkovi contained highest vitamin C content of 1320 ppm but lower content was found in 10 hoblies viz. Hiregunturu, Imangala, Kasaba, Ramagiri, Mangaluru, Mudabidre, Ajekaru, Karkala, Kundapura and Vadse wherein its concentration was only 264 ppm. Highly significant variations existed in vitamin C content among all the 35 taluks of 6 districts (Table 4). Mean over all the taluks indicated that highest vitamin C content (1012 ppm) was in Davanagere district followed by Shimoga and Chikamagaluru districts (897 ppm). Similarly, Areca nut Research and Development Foundation (ARDF 2015), Sirsi have reported that, vitamin-A and vitamin B-6 were found in traces in areca nut samples of Karnataka.

The determination of vitamin B6, it should be noted that vitamin B6 are usually unstable and therefore the reference and sample protocols must be handled with great care while using HPLC. Separation and quantification should be done with a high level of precision and suitable methods. HPLC method for analysing vitamin B6 is common but a single run HPLC method for simultaneous analysis of vitamins are undocumented. It is therefore important to have a single most sensitive HPLC method, which is robust, rapid and efficient for determining all the water-soluble vitamins in a single run. Both hobli wise and taluk wise areca samples recorded highly significant variations in vitamin B6 contents with a range of 91.33 to 10.00 ppm. Vitamin C content in areca nut was determined by the process of titration. Multiple tests were carried out in areca nut samples collected from different districts of Karnataka. Vitamin C content varied in different districts and hoblies of Karnataka with a range of 264-1408 ppm on dry weight basis. The variability in vitamins content may be due to difference in climatic conditions, varietal change, soil types and areca nut storage conditions. Also, during wet processing of areca nut the product is subjected to boiling processes and varieties of ingredients are added to impart good colour and texture (Table 5). The farmers make use jamun tree bark, jaggery, co oking oil, kaachu, lime and many more cheaply available substances for wet processing, which may also cause differences in vitamin contents.

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