BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2017. Vol. **14**(2), p. 691-695

Studies on Variation in Arecoline Content of Arecanuts Collected from Different Parts of Karnataka

B. R. Gurumurthy, H. C. Swathi, J. Sahana and S. P. Nataraj

Department of Crop physiology, University of Agricultural and Horticultural Sciences, Navile, Shivamogga-577225, India.

http://dx.doi.org/10.13005/bbra/2496

(Received: 07 April 2017; accepted: 19 April 2017)

Arecoline is a nicotinic acid-based alkaloid found in the areca nut, the fruit of the areca palm (*Areca catechu*). It is an oily liquid that is soluble in water, alcohols, and ether. HPLC method is simple and rapid for determination of arecoline content in areca nut. Areca samples were collected from Shimoga, Davanagere, Chikkamagalur, Chitradurga, Dakshina kannada and Udupi districts of Karnataka, India. The collected areca samples were powdered and arecoline is extracted from samples collected from different hoblies of the districts. The extraction method was optimized to obtain pure arecoline before analysis to separate any interference in order to maximize the specificity and sensitivity of the method. The regionwise arecoline content has been compared. The concentration of arecoline varied from area to area depending on environmental factors, differential processing methods, age of the plantations, varietal differences etc.

Keywords: Areca nut, Arecoline, HPLC.

Areca nut (*Areca catechu* L. *Palmaceae*) a tropical fruit, also called betel nut is widely distributed in different parts of the world. Areca nut is widely grown in South East Asia. About 90 % of the areca is used as commercial preparations in a large scale. The processing involves dehusking of fruits, removing nuts, processing and sun drying or with artificial heat or sometimes smoking. The dried product is graded according to harvest, color, shape, and size. Areca nut contains alkaloids, which are arecaidine, arecoline (methyl ester of arecaidine), guvacine, guvacoline (methyl ester of guvacine), arecolidine, homoarecoline and some other minute amount of similar alkaloids. Arecoline is the main alkaloid found in the nut. In herbal medicine, the arecanut has been used to treat parasitic worms since it contains tannins. The arecanut extract also shows potential pharmaceutical activities such as analgesic, anti inflammatory and antioxidant. Unfortunately, arecoline has also shown genotoxic, mutagenic and carcinogenic effects. It is therefore important to determine the content of arecoline in areca nut extract before use in pharmaceutics or cosmetics. The extraction efficiency of the traditional extraction method was also low. Previously, few quantitation methods have been reported for determination of arecoline content, these include ultraviolet (UV) spectrophotometry, gas chromatography (GC) and high performance liquid chromatography (HPLC). The HPLC technique is usually considered to be easier than GC and more sensitive and selective than UV spectrophotometry. A reverse phase HPLC method for the determination of arecoline was developed by Aromdee *et al.* to determine the contents of arecoline in different shapes of areca

* To whom all correspondence should be addressed.
E-mail:swathicng@gmail.com

nut. Cox *et al* also successfully employed HPLC for the determination of arecoline in human saliva. In the present study, HPLC method has been standardized for estimation of arecoline in areca samples for determination of arecoline content in areca samples obtained from different areas of Karnataka. The extraction method was optimized to obtain pure arecoline before analysis to separate any interference in order to maximize the specificity and sensitivity of the method. The contents of arecoline in different areas of areca nuts were compared.

MATERIALS AND METHODS

Areca samples were collected from different districts of Karnataka (Shimoga, Davanagere, Chikkamagalur, Chitradurga, Udupi and Dakshina Kannada). An UHPLC model ultimate 3000, thermoscientific with pump, autosampler and UV detector has been employed for the study. An hypersil gold C18 column used for the study. The flow rate was 1.0 ml min^{-1}.Milipored water has been used for mobile phase and standard preparation. Different Solvents, Acetonitrile (solvent phase) and arecoline hydrobromide (standard) were of HPLC grade.

Sample Preparation

The areca samples were finely powdered and ground in methanol in the ratio of 1.0 g: 2.5 ml. The suspension was filtered through a suction filter. 10 ml of the filtrate was dried in vacuum, further 2 ml of 0.1 N HCL was added and transferred into a 15 ml test tube. The acidic solution was washed with 5 ml of chloroform. An aliquot of 1 ml of the acid layer was made alkaline with 0.5 ml of 1N ammonium hydroxide. Chloroform added and vertexed for 3 minutes. One ml of the chloroform extract was evaporated, then one ml of the mobile phase was added, shaken well and filtered through a membrane filter. The solution (20µl) was injected into the equilibrated HPLC system. 1 ml of 1 mg/ml arecoline hydrobromide solution was spiked into 10 ml of the methanol solution of the sample in order to verify the accuracy of the method, then extracted and injected.

Standard preparation

A stock standard solution of 1.0 mg ml^{-1} arecoline hydrobromide was prepared. A series of standard solutions were prepared from 25, 50, 75, 100 and 200 of the stock solutions and diluted to 10 ml with the mobile phase. For daily use, only solutions containing 25, 50 and 100µl of the stock solution were prepared.

Mobile phase

Potassium dihydrogen phosphate (10g) was dissolved in 990 ml of water and 3.5 ml of phosphoric acid, 8 ml of triethylamine and 9 ml of acetonitrile have been added & mixed well and the pH was adjusted to 5.5 with triethylamine or phosphoric acid.

RESULTS AND DISCUSSION

The optimal wavelength for arecoline detection at 215 nm was established using UV absorbance scans over the range of 200-400 nm. Although detection at 215 nm was more sensitive and also showed high signal response and did not pose a problem in terms of co-elusion with impurities and solvent used. The flow tare of mobile phase was ml min^{-1} and the retention time of arecoline was rather short (within 7 min). Generally, the retention time of arecoline might be adjusted by altering the composition of mobile phase and pH. If the ratio of acetonitrile is increased, the retention time of arecoline decreased and the peak was sharper. If the pH of the mobile phase increased, the retention time of arecoline would be increased due to the presence of more non-ionized form. The retention time for determination of arecoline is long enough (7 min), this developed method might be able to use further for the determination of arecoline in plasma and pharmaceutical formulation since most proteins and pharmaceutical vehicle interferences are usually eluted in a reverse-phase chromatographic system within 5-6 min. The mobile phase used in this study was simple containing a phosphate buffer and acetonitrile without any modifiers, and easy to prepare.

Analytical results obtained from thalukwise samples in each district has been presented in the table-1

There was a significant difference in arecoline content in different thaluks of Shimoga district. High arecoline content was observed in Bhadravathi taluk (591.40 ppm) followed by Hosanagara taluk (550.80 ppm) and the less

arecoline content was observed in the Thirthahalli taluk (121.60 ppm) followed by Shikaripura taluk of shimoga district (258 ppm).

The contents of arecoline in Chikkamagalur and Kadur were 517.40 and 316.40 ppm respectively. Arecoline content was less in Tarikere (114.60 ppm) and Sringeri (215 ppm).

There was no much significant difference of arecoline content in Davanagere district. In Honnali and Jagalur taluks the arecoline content was 381.40 and 333.20 ppm respectively. 169.60 and 182.40 ppm were observed in Harihara and Channagiri taluks. The same was observed in Chitradurga district, where in no significant differences were observed in arecoline contents.

Arecoline content varied in different taluks of Dakshina kannada and Udupi districts. High concentration of arecoline content was observed in Belthangadi (1023.80 ppm) followed by Bantwala (379.80 ppm) and less concentration of arecoline was observed in Sullia (229.80 ppm). In Udupi district arecoline content was more in Brahmavara and less was in Baindur.

The contents of arecoline in hoblies in different districts of Karnataka were compared (Table 2). There was a significant difference in arecoline content between among hoblies of Shimoga district. Nidige and Kasaba hoblies of Shimoga taluk had higher concentration of arecoline (1230.67 and 967.67 ppm) compare to the other hoblies. Low concentration of arecoline was determined in the Holehonnur of Bhadravathi taluk (46.67 ppm) and Kasaba hobli of Shikaripura taluk (73.67 ppm) of Shimoga district.

The samples of Ambale and Avathi hoblies of Chikkamagalur taluk recorded more arecoline content (818.33 and 785.67 ppm). Banakal hobli of Mudigere taluk contained low arecoline (109 ppm) followed by kasaba hobli of sringeri taluk (111.33 ppm).

In govinkovi hobly of Honnali taluk, higher concentration of arecoline (867 and 813.67 ppm) was observed and low concentration, were in Malebennur (109.33 ppm) and Basavapatna (127 ppm) hoblies of Davanagere District.

Significant difference were observed in arecoline contents, among different hoblies of Chitradurga, The arecoline was high in kasaba hobli and low concentration of arecoline were recorded in the Imangala and Kasaba hoblies of Hiriyur and Holalkere taluks (101 ppm and 161.33 ppm) respectively.

The hoblies of Belthangadi taluks of Dakshina kannada district were having higher

Table 1. Arecoline content (ppm) in Different thaluks. (District X Thaluks) of Karnataka

Districts	Shimoga	Chikkamagalur	Davanagere	Chitradurga	DK	Udupi
1.	378.8 (Shimoga)	517.4 (Chikkamagalur)	182.4 (Chennagiri)	491.8 (Chitradurga)	379.8 (Bantwala)	362.2 (Brahmavara)
2.	####### (Bhadravathi)	316.4 (Kadur)	362.6 (Davanagere)	310.6 (Hiriyur)	1023.8 (Belthangadi)	84 (Baindur)
3.	####### (Sagar)	243.8 (Koppa)	169.6 (Harihara)	340.6 (Holalkere)	323 (Kadaba)	210.8 (Karkala)
4.	####### (Hosanagar)	215 (Sringeri)	381.4 (Honnali)	313.8 (Hosadurga)	350 (Mangaluru)	281 (Kundapura)
5.	####### (Thirthahalli)	268.4 (Mudigere)	333.2 (Jagalur)	-	246 (Mudabidre)	155.8 (Udupi)
6.	####### (Sorab)	251.8 (N R Pura)	-	-	370.6 (Puttur)	-
7.	####### (Shikaripura)	114.6 (Tarikere)	-	-	229.8 (Sullia)	-
Mean	359.5143	275.3429	285.84	364.2	417.5714	218.76
P 0.01	NS	**	NS	NS	NS	**
CV	55.23774	52.79056	48.15244	48.26275	44.09953	60.40468
S.Em±	88.81105	65.00475	61.55401	78.60805	82.35306	59.09538

concentration of arecoline (1444.67 and 743.67 ppm) and Mangalore A and Mulki hoblies of Mangalore taluk had low concentration of arecoline (162.33 and 175 ppm).

In Udupi district arecoline concentration was low in Baindur (83.67 ppm) and Kapu (84.67 ppm) hoblies of Baindur and Udupi taluks. Kota and Karkala recorded lower concentration of arecoline (527 and 377 ppm).

Aromdee *et al*. (2003) reported the arecoline in the arecanut depends on the age, season, location, management practices and other

Table 2. Variability in arecoline contents (ppm) in different hoblies in areca growing districts of Karnataka

Districts Hoblies	Shimoga in ppm	Chikkamagalur in ppm	Davanagere in ppm	Chitradurga in ppm	DK in ppm	Udupi in ppm
1.	221.67	818.33	226.33	969.00	610.67	527.00
2.	293.00	558.00	139.67	356.67	438.67	114.67
3.	250.33	785.67	239.00	332.33	205.00	83.67
4.	163.33	295.67	127.00	443.67	1444.67	100.00
5.	967.67	129.33	180.33	101.00	743.67	377.00
6.	280.67	370.67	120.00	334.00	323.00	351.00
7.	400.67	401.33	279.00	534.33	657.00	156.00
8.	1230.67	408.00	467.00	249.00	162.33	203.00
9.	115.33	342.67	321.00	161.33	260.67	84.67
10.	351.00	319.67	210.33	175.67	175.00	-
11.	691.67	110.33	109.33	442.00	494.67	-
12.	835.33	474.33	250.67	481.67	245.67	-
13.	46.67	212.00	813.67	335.67	292.67	-
14.	354.33	103.67	467.00	281.67	487.00	-
15.	214.00	360.33	169.67	-	246.67	-
16.	459.67	135.33	205.00	-	204.00	-
17.	75.00	229.00	288.33	-	-	-
18.	361.00	191.33	268.00	-	-	-
19.	871.33	251.00	431.33	-	-	-
20.	632.33	111.33	-	-	-	-
21.	281.33	394.67	-	-	-	-
22.	335.67	331.67	-	-	-	-
23.	170.00	313.00	-	-	-	-
24.	77.00	160.33	-	-	-	-
25.	110.33	109.00	-	-	-	-
26.	140.67	123.33	-	-	-	-
27.	385.67	-	-	-	-	-
28.	155.67	-	-	-	-	-
29.	271.33	-	-	-	-	-
30.	560.00	-	-	-	-	-
31.	245.67	-	-	-	-	-
32.	590.00	-	-	-	-	-
33.	375.33	-	-	-	-	-
34.	208.67	-	-	-	-	-
35.	316.33	-	-	-	-	-
36.	317.33	-	-	-	-	-
37.	73.67	-	-	-	-	-
Mean	372.1802	309.2308	279.614	371.2857	436.9583	221.8889
P 0.01	**	**	**	**	**	**
CV	0.585875	0.373411	0.591856	0.254359	1.328772	2.613207
S.Em±	1.227806	0.666667	0.955465	0.54525	3.352199	3.347717

factors involved in the production of arecoline in areca nuts. Arecanut grown in different areas might contain different amounts of arecoline.

CONCLUSION

This study contributes to the establishment of an easy and rapid HPLC method for the determination of arecoline extract in arecanut samples. The HPLC method was found to be simple, rapid, precise, accurate and selective. In addition, the specific extraction procedure for arecoline described in this study assists the ease in HPLC analysis. In summary, the proposed method can be used for arecoline analysis extracted from dried areca nuts. The concentration of arecoline content varies from area to area, this is because of the determination of arecoline in these nuts should be more selective according to the location of the plantation, and other factors involved in the production of arecoline in areca nuts.

REFERENCES

1. Aromdee, C., Panuwongse, S., Anorach, R. And Vorarat, A high pressure liquid chromatographic method for the determination of arecoline in areca nuts. *Thai J. Pharm. Sci.,* 2003; **27**: 41-7.
2. Cox, S., Piatkov, I., Vickers, E. R. And Ma, G., High-performance liquid chromatographic determination of arecoline in human saliva. *J. Chromatogr.,* 2004; **1032**: 93-95.