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Variability in Polyphenol Content in Areca Nut (*Areca catechu*) Samples of Karnataka

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The phenolic compounds or polyphenols, secondary metabolites of plants, are phytochemicals that exhibit antioxidant activity and consequently possess a beneficial physiological effect (Bravo, 1998). Plant polyphenolic compounds have been recognised for their ability to prevent oxidation of susceptible substances by virtue of their electron donating property due to presence of large number of phenolic hydroxyl groups. The seed of *Areca catechu* contains higher proportions of polyphenolic compounds mainly tannins (Zhang *et al.,* 2008). In India 7.06 lakh ton of arecanut is produced annually (2016-17), major portion of which (App, 90%) utilised for human consumption. Hence it is essential to study variability in polyphenol contents of areca samples grown under diverse conditions and its impact on consumers. In the present study 850 areca samples were collected from 6 important area growing districts of Karnataka and further hobliwise pooling was done before polyphenol content was determined. In Shimogga district high concentration of polyphenol was found in Bhadrvathi thaluk (3.02%) followed by Shimogga (2.68%) and less concentration was found in Sorab taluk (1.17%). Polyphenol content varies from 0.82 % to 2.14 % in Chikkamagalur. The highest concentration was observed in Tarikere (2.14%) and less in Kadur (1.55%) . In Davanagere district polyphenol content was highest in Channagiri (2.50%) followed by Jagalur (1.29%). The results of total polyphenol content in different district are very different. But the regionwise variability was not noticed.

Keywords : Areca nut, Polyphenol, Folin-Ciocalteu reaction.

Medicinal plants are the richest source of bioactive compounds used in traditional and modern medicine. The research on phenolic compounds has been growing lately because of the increasing worldwide request for phenolic compounds and their increasing application in food industry. The beneficial effects derived from phenolic compounds have been attributed to their antioxidant, anticancer and superoxide radical scavenging activity (Djeridane *et al*., 2006). The Areca nut is commonly known as betel nut. The chemical constituents of Areca nut are mainly polyphenols including tannins, flavonoids and alkaloids. Polyphenols are functional compounds in plants, which possess many bioactivities beneficial for humans. polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages, fruits like

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grapes, apple, pear, cherries. The aim of this study is to establish a highly efficient method for extracting polyphenol compounds from areca seeds and quantification of total polyphenol content based on colorimetric measurement. Total phenols can be measured using the Folin-Ciocalteu reaction (Han *et al.,* 2010). Polyphenols in plant extracts react with specific Folin-Ciocalteu reagent to form a blue complex that can be quantified by visible-light spectrophotometry. Polyphenols are the large numbers of natural phenolic compound found abundantly in plants, possessing antioxidant activity. They are diverse in their chemical structure, nature and biological activity, capable of providing protection against oxidative stress and thus may plays a significant role in the prevention or improvement of several clinical conditions like:cancer, osteoporosis, neurodegenerative, cardiovascular diseases, diabetes mellitus etc. Owing to their involvement in human consumption, regulation of physiology and its utility in control of many ailments, it is highly essential to understand the content of polyphenols in areca samples and regionwise variability.

MATERIALS AND METHODS

Sample collection

Areca nut samples were collected from different districts of Karnataka (shivamogga, Davangere, Chikkamagalur, Chitradurga, Udupi, and Dakshina kannada). The total phenolic content in araeca nut samples was determined by Folin-Ciocalteu method.

Extraction of Polyphenol from Areca Seed

Maximum polyphenols (407.47 mg GAE g-1), total tannin and its antioxidant activity with minimum arecoline (1.73 mg g-1 of sample) was achieved by using 80% acetone at pH 4 for 90 min with 10% w/v substrate under shaking conditions (chavan and singhal, 2013). To determine the antioxidant compounds, the optimum extraction conditions were used. 5 g of the finely powdered and dried areca seed sample was extracted using 55 ml of 70% ethanol at 70°C for 120 min by reflux. The extracts were filtered through Whatman No. 4 paper under reduced pressure, and then lyophilized by LGZ-10D Freezer Dryer. All the samples were redissolved in 70% ethanol at a concentration

Table 1. Thalukwise variability in polyphenol content (%) in selected districts of Karnataka

Districts Udupi ~~Thaluks~~	Shimoga	Chikkamagalur	Davanagere		Chitradurga	Dakshina Kannada
1.	2.68(Shimoga)	1.00(Chikkamagalur)	2.50(Chennagiri)	3.24(Chitradurga)	2.86(Bantwala)	1.47(Brahmavara)
2.	3.02(Bhadravathi)	0.82(Kadur)	0.59(Davanagere)	1.67(Hiriyur)	2.16(Belthangadi)	2.43(Baindur)
3.	1.84(Sagar)	1.55(Koppa)	0.88(Harihara)	1.89(Holalkere)	4.21(Kadaba)	1.38(Karkala)
4.	2.59(Hosanagar)	1.07(Sringeri)	1.08(Honnali)	3.35(Hosadurga)	0.94(Mangaluru)	2.38(Kundapura)
5.	2.56(Thirthahalli)	1.93(Mudigere)	1.29(Jagalur)	-	5.53(Mudabidre)	1.53(Udupi)
6.	1.17(Sorab)	0.85(N R Pura)	-	-	–	-
7.	2.14(Shikaripura)	2.14(Tarikere)	-	-	–	-
P 0.01	**	**	**	**	**	**
CV	2.13	2.58	1.91	1.50	0.72	1.46
S.Em±	0.02	0.01	0.010	0.01	0.01	0.01

Note : Name of thaluks are indicated in parenthesis.

Table 2. Hoblewise variability in polyphenol content (%) in selected districts of Karnataka

Districts Shimogga	Hoblies	Chikkamagalur	Davanagere	Chitradurga	Dakshina Kannada	Udupi
1.	3.09(Bhadravathi)	1.11(Ambale)	1.91(Basavapattana)	1.82 (Baramasagara)	3.50(Belathangadi)	1.45(Ajekar)
2.	3.60(Holehonnur)	0.84(Chikkamagalur)	1.81(Channageri)	6.07 (Chitradurga)	2.23(Kokkada)	1.52(Karkala)
3.	2.79(Kudligere)	0.93(Avuthi)	0.96(Santhebannur)	2.02 (Hireguntanur)	1.94(Venoor)	2.43(Byndoor)
4.	2.90(Hosanagar)	0.74(Jagara)	1.50(Ubrani)	3.10 (Hiriyur)	2.42(Bantwala)	1.81(Kundapur)
5.	3.57(Humcha)	1.31(Kandya)	8.15(Anagodu)	1.82 (B. Durga)	1.00(Panemangalore)	0.93(Wandse)
6.	3.04(Kerehalli)	0.94(Lakya)	0.84(Davanagere)	1.57 (Holalkere)	3.22(Vitla)	2.72(Brahmavar)
7.	2.98(Nagara)	0.99(Vastare)	0.74(Mayakonda)	2.37 (Ramgeri)	1.71(Mangalore)	2.05(Kapu)
8.	2.52(Anandapuram)	0.91(Hirenallur)	0.95(Harihara)	0.93 (Thalya)	1.22(Gurupura)	1.37(Kota)
9.	2.50(Barangi)	1.01(Kadur)	0.84(Malebennur)	1.64 (Hosadurga)	13.90(Moolki)	1.70(Udupi)
10.	2.89(Avinahalli)	0.96(Beerur)	0.95(Arasikere)	2.48 (Madadakere)	0.91(Surath kal)	-
11.	2.72(Karoor)	1.18(Sakrepatna)	1.64(Chigateri)	1.64 (Mathodu)	10.09(Moodabidri)	-
12.	3.44(Sagar)	1.79(Singhatagere)	0.57(Telagi)	4.94 (Aimangala)	1.04(Kadaba)	-
13.	3.30(Talaguppa)	1.52(Yagati)	0.91(Belagutti)	1.78 (Dharmapura)	1.30(Puttur)	-
14.	2.29(Anjanapura)	1.41(Hariharapura)	1.21(Govinakovi)	-	-	-
15.	4.11(Hosur)	1.02(Koppa)	0.88(Honnali)	-	-	-
16.	1.80(Udagani)	1.16(Gonibeedu)	1.70(Saswehalli)	-	-	-
17.	1.64(Shikaripura)	1.74(Balehonnur)	-	-	-	-
18.	0.91(Talagunda)	2.56(Narasimharajpur)	-	-	-	-
19.	2.63(Haranahalli)	1.51(Kigga)	-	-	-	-
20.	1.92(Kumsi)	0.75(Sringeri)	-	-	-	-
21.	3.19(Nidige)	0.91(Ajjampura)	-	-	-	-
22.	2.42(Shimoga)	1.91(Amruthapura)	-	-	-	-
23.	2.12(Anavatti)	2.41(Lakkavalli)	-	-	-	-
24.	3.11(Chandragutti)	-	-	-	-	-
25.	0.92(Jade)	-	-	-	-	-
26.	1.38(Kuppagadde)	-	-	-	-	-
27.	1.30(Sorab)	-	-	-	-	-
28.	1.05(Ulavi)	-	-	-	-	-
29.	1.91(Agrahara)	-	-	-	-	-
30.	1.70(Agumbe)	-	-	-	-	-
31.	2.79(Mandagadde)	-	-	-	-	-
Mean	2.47	1.28	1.59	2.47	3.39	1.77
P 0.01	**	**	**	**	**	**
CV (%)	8.77	7.15	7.26	6.71	5.11	9.47
S.Em±	0.12	0.05	0.06	0.09	0.10	0.09

Note : Name of hoblies are indicated in parenthesis

Table 3. List of main 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				

of 5.0 mg/ml and analyzed for their content of polyphenols.

Determination of Polyphenol Content

Total phenolic content of the extracts were determined by the Folin-Ciocalteu method. phenolic content of the extracts was estimated using a colorimetric assay. 1 ml of sample was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and the volume was adjusted to 10 ml with distilled water. The tubes were vortexed for 15 seconds. The reaction was kept in dark for 90 min. The absorbance was then read at 725 nm in UV- VIS spectrophotometer against blank. Catechin was used to produce a standard curve (2.0–12.0 ¼g/ml; $y = 0.0631x$ " 0.0611; $R2 = 0.9992$).The results were expressed as mg of catechin equivalents/g of extract. The experiment was conducted in triplicate and the mean value was used for interpretation of the data.

RESULTS AND DISCUSSION

Results indicating thalukwise variability in polyphenol content (%) in important areca growing districts of Karnataka has been presented in the table.1 Highest mean polyphenol was recorded in Mudabidre thaluk (5.53 %) followed by Kadaba (4.21%) and Hosadurga (3.35 %) thaluks. Lowest polyphenol has noticed in Harihara (0.88 %) and karkala (0.93 %) thaluks of Dakshina kannada districts. Differences in polyphenol contents were highly significant in the thaluks of all the 6 districts.

Mathew and Govindarajan observed during 1963, that the polyphenol of arecanut at all maturity stages are mainly flavonoids and decrease in concentration with maturity on dry weight basis. The pattern of changes with maturation and ripening is due to insolubilization of higher polymers together with the formation of fresh monomers and intermediate polymers.

Further, mean polyphenols values obtained from pooled areca samples from hoblies are depicted in table 2. A highly significant variability was noticed in all the districts under study. Highest polyphenol was recorded in Surathkal hobli (13.9 %) followed by Uppinangadi (10.09 %) and Santhebanur (8.15 %). The vast

variability in polyphenol content in areca samples can be attributed to methods of wet and dry processing and diverse range of materials used by the growers to obtain high grade wet processed areca (Table 3). During wet processing farmers of Shimoga, Chikkamagalur, Davangere and Chitradurga commonly make use of jagerry, cooking oil, jamun tree bark and betel leaf. Some farmers of shimoga district make use of lime and 13 different cheaply available materials for wet processing with the objective of imparting good colour and shining to the finished products. In Davangere people use lime, kaachu, sandal wood, pongamia and eucalyptus for the processing where as, the growers of Chitradurga additionally use betel leaves and areca powder.

CONCLUSION

Total phenolic content in areca nut sample was determined with Folin-Ciocalteu method. The Folin–Ciocalteu reagent is sensitive to reducing compounds, polyphenols and thus produces a blue colour complex. The UV-Vis spectrophotometric method was suitable for the determination of polyphenol content. The pooled data of all the samples collected from the hoblies of different districts of Karnataka indicated that contents of polyphenols varied significantly with range of 0.83 to 6.20 %. However regionwise variability could not be recorded in the samples in the selected study areas. The variability may be due to different factors such as the age of plantations, variety geographic area, processing methods, materials used for processing and variations in sunlight exposure, methods of cultivation and the fertilizers used.

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