

Estimation and Comparison of Copper Content in Raw Areca Nuts and Commercial Areca Nut Products: Implications in Increasing Prevalence of Oral Submucous Fibrosis (OSMF)

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ABSTRACT

Background: Oral Squamous cell carcinoma (OSCC) is often preceded by potentially malignant disorders (PMDs) like oral submucous fibrosis (OSMF). The rate of transformation of OSMF to OSCC ranges from 3 to 19%. OSMF is etiologically related to chewing of areca nut (betel nut), a habit prevalent among the population groups in south-east Asia. Along with alkaloids, the high copper content in areca nut plays an important role in the pathogenesis of OSMF. The increased prevalence of OSMF in the last two decades or so corresponds with the increased processing and commercialization of areca nut products.

Aim: The aim of the study was to estimate and compare the copper content of raw areca nuts in three different stages of maturity, and commercial areca nut products.

Materials and Methods: Raw areca nut samples of three

different maturities were obtained from four plantations in Sullia, Karnataka, India and commercial areca nut products were obtained from local shops in Chidambaram, Tamil Nadu, India. The samples were grounded and subjected to Atomic Absorption Spectrometry (AAS) for copper analysis.

Results: There was statistically significant difference in copper content in raw areca nuts of all three maturities ($p < 0.05$) and was highest in the exfoliated mature nuts. Importantly copper level was significantly higher in the commercial products compared to raw areca nuts of different degrees of maturity ($p < 0.05$).

Conclusions: The copper levels in commercial products are significantly higher than that of raw areca nuts in all three stages of maturity. The increase in copper content on processing and post commercialization can be related to the increasing prevalence of OSMF.

Keywords: Absorption Spectrometry (AAS), Areca nut, Oral submucous fibrosis

INTRODUCTION

Oral cancer ranks from the sixth to eighth most common cancer around the world, with a significant difference in incidence among countries [1]. Data from population based registries under the National Cancer Registry Programme (NCRP) indicate that in India, oral cancer is the most prevalent cancer in males and the third most prevalent in females, indicating a major health problem constituting up to 40% of all cancers [2]. OSCC is the most common cancer of the oral cavity and represents about 90% of all oral malignancies [3]. Most OSCC are usually preceded by certain changes in oral mucosa. These warning lesions are referred as PMDs. OSMF is one of the established PMD found in oral mucosa [4]. The malignant transformation (to OSCC) of the disorder ranges from 3 to 19% [5].

Areca nut has been chewed for hundreds of years and is currently being used by 10% of the world's population [6]. Areca nut chewing has been etiologically related to OSMF. Along with alkaloids in areca nut, the high copper content in the nut is believed to play an important role in the pathogenesis of OSMF [7,8]. It is suggested that substantial amounts of copper is released into saliva on chewing areca products and is absorbed into oral mucosa [9]. Copper acts by up regulating lysyl oxidase activity, which is the key enzyme in collagen metabolic pathway. Thereby copper enhances collagen synthesis by fibroblasts, facilitates its cross linking and, eventually, inhibits its degradation [10,11]. Several studies have reported the increased serum and tissue copper levels in OSMF patients [7,8,10].

India is the largest producers of areca nut, with the South Indian states of Karnataka, Kerala and Tamil Nadu contributing to the bulk of it [12]. With the introduction of various commercial areca nut products with or without tobacco and other ingredients in the market, the raw areca nut chewing habit has decreased. The increase in prevalence of OSMF in the last two decades or so is coinciding with the increased processing and commercialization of areca products since the early 1980s [13]. The number of young patients with OSMF who is exposed to areca nut products only for a short duration also increased substantially during this period [14]. However, the reason for increased prevalence of the disorder in India during the last two decades is not well explained as areca nut was in use since many centuries. In an attempt to explain this, we estimated the copper content in raw areca nut and its processed forms.

MATERIALS AND METHODS

Raw areca nut samples were obtained from four plantations in Sullia Taluk of Dakshina Kannada district, Karnataka, India. Areca nut obtained from each plantations was categorized depending on the degree of maturity into unripe (green), ripe (yellow-orange) and exfoliated mature nuts [Table/Fig-1,2,3]. The unripe and ripe areca nuts were plucked from the palms; where as exfoliated ones were collected from the surroundings of palm. Raw areca nuts from these areas are distributed all over South India and are used in the commercial preparations.

Samples of the commercial products of areca nut and were

obtained from local shops in Chidambaram, Tamil Nadu, India. Two different brands of commercial areca nut products were collected from 4 outlets in various parts of the city. Samples of the same batch numbers were mixed together to obtain a representative sample of that product.

All samples were ground and homogenised using a mortar and pestle and was stored in clean polythene bags. Test portions were dried and then ashed at 450°C with initial temperature not higher than 100° C which was gradually increased ($\leq 50^{\circ}\text{C/h}$). 5 mL of 6M hydrochloric acid (HCl) was added to the ash and the solution was evaporated to dryness. The residue was dissolved in 0.1M Nitric acid (HNO₃) and the resulting solution was analyzed using flame atomic absorption spectrometry (FAAS). The spectrophotometer was standardized using the standard solution of the element being analyzed and acidified deionized water was aspirated to zero the instrument. Acetylene flame was used and sample extracts were aspirated. The absorption of radiation by copper from the sample solutions was measured in wavelengths and its concentration was read-off by the instrument. The obtained results were expressed in mg.kg⁻¹.

STATISTICAL ANALYSIS

The data obtained was subjected to a statistical analysis and basic variation statistical values (arithmetic mean, standard deviation, standard error, maximum and minimum value) were calculated. The differences between means were calculated by a one-way analysis of variance and multiple comparisons were done using Post Hoc test Tukey HSD at significance level $p < 0.05$.

RESULTS

The copper content was determined in the 36 samples obtained from 4 plantations and 6 samples of commercial products. The average copper content for various groups of raw areca nut and commercial products are shown in [Table/Fig-4]. This analysis has yielded very useful information regarding the copper content in areca nuts of different degrees of maturity. Among raw areca nut, copper content was least in the unripe areca nut (3.64 ± 0.84 mg.kg⁻¹) and was highest in the exfoliated mature areca nut (8.77 ± 0.65 mg.kg⁻¹). Ripened areca nut showed intermediate values (7.84 ± 0.81 mg.kg⁻¹) [Table/Fig-4]. There was statistically significant difference in copper content of areca nuts in all three stages maturity ($p < 0.05$) [Table/Fig-5 and 6].

The copper level in commercial products was higher (11.39 ± 1.02 and 12.83 ± 0.63 mg.kg⁻¹) than the raw areca nut [Table/Fig-4]. The difference in copper content between commercial products and raw areca nut of all maturities was also statistically significant ($p < 0.05$) [Table/Fig-5 and 6].

DISCUSSION

In the present study, copper content increased significantly with maturity in raw areca nuts. Jayalakshmi & Mathew (1982) [15] have mentioned about the difference in moisture content in the unripe and ripe areca nut. According to their observation the moisture content in unripe areca nut was about 69.4–74.1 % and that of ripe ones was 38.9–56.7%, suggesting that the moisture content was getting reduced as the nut matures. In our study, the unripe nuts which are rich in moisture content showed the least copper levels for a given weight. The exfoliated mature nuts with relatively less moisture content showed higher amounts of copper.

Similarly, the copper content in commercial products was significantly higher than the raw areca nut. Studies by Shakya S et al [16] (14.9–18.3 mg kg⁻¹) and Trivedy C et al., [9] (18 ± 8.7 mg kg⁻¹) also revealed higher copper levels in commercial areca nut products. Dhaware et al., [17] in their study stated that among the smokeless tobacco products, higher copper content was observed in the commercial preparations containing areca nut.

During processing of areca nut for commercial purpose, the moisture content is further reduced by drying or roasting. Understandably, the nut becomes more concentrated with its mineral and chemical constituents. This increase in mineral concentration of nut on processing, will probably explain the higher copper content in the commercial areca nut products. The dry weight of nut also reduces significantly along with volumetric shrinkage as it loses the moisture content during the processing. Thus in effect, individuals using commercial products will be consuming areca nut which is rich in copper compared to those who use raw areca nut.



[Table/Fig-1]: Unripe areca nuts



[Table/Fig-2]: Ripe areca nuts



[Table/Fig-3]: Mature exfoliated areca nut

Raw areca nut groups and commercial products	N	Mean copper content in mg.kg ⁻¹	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference	
					Lower	Upper
Unripe	12	3.6417	0.83656	0.24149	3.1101	4.1732
Ripe	12	7.8408	0.80622	0.23274	7.3286	8.3531
Exfoliated	12	8.7675	0.64677	0.18671	8.3566	9.1784
Product1	3	11.3900	1.02220	0.59017	8.8507	13.9293
Product2	3	12.8333	0.63375	0.36589	11.2590	14.4076

[Table/Fig-4]: Descriptive Statistics- Showing the mean copper levels in each group

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	330.032	4	82.508	136.636	.000
Within Groups	22.343	37		.604	
Total	352.375	41			

[Table/Fig-5]: One way ANOVA
Dependent Variable: Copper

(I) group vs (J) groups		Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Unripe	Ripe	-4.19917*	.31724	.000	-5.1086	-3.2897
	Exfoliated	-5.12583*	.31724	.000	-6.0353	-4.2164
	Product 1	-7.74833*	.50160	.000	-9.1863	-6.3103
	Product 2	-9.19167*	.50160	.000	-10.6297	-7.7537
Ripe	Unripe	4.19917*	.31724	.000	3.2897	5.1086
	Exfoliated	-.92667*	.31724	.044	-1.8361	-.0172
	Product 1	-3.54917*	.50160	.000	-4.9872	-2.1112
	Product 2	-4.99250*	.50160	.000	-6.4305	-3.5545
Exfoliated	Unripe	5.12583*	.31724	.000	4.2164	6.0353
	Ripe	.92667*	.31724	.044	.0172	1.8361
	Product 1	-2.62250*	.50160	.000	-4.0605	-1.1845
	Product 2	-4.06583*	.50160	.000	-5.5038	-2.6278
Product 1	Unripe	7.74833*	.50160	.000	6.3103	9.1863
	Ripe	3.54917*	.50160	.000	2.1112	4.9872
	Exfoliated	2.62250*	.50160	.000	1.1845	4.0605
	Product 2	-1.44333	.63448	.176	-3.2623	.3756
Product 2	Unripe	9.19167*	.50160	.000	7.7537	10.6297
	Ripe	4.99250*	.50160	.000	3.5545	6.4305
	Exfoliated	4.06583*	.50160	.000	2.6278	5.5038
	Product 1	1.44333	.63448	.176	-.3756	3.2623

[Table/Fig-6]: Multiple Comparisons with in groups and between groups
Dependent Variable: Copper
Tukey HSD
*The mean difference is significant at the 0.05 level

It is quite clear from the above observations that the copper absorbed to oral mucosa on chewing commercial products will be significantly higher. This may be related to the increased prevalence of OSMF after the launch of commercial areca nut products. Highly concentrated areca nut products may also be responsible for the onset of OSMF in younger patients with relatively shorter duration of chewing habit.

CONCLUSIONS

By modifying the processing methods, products similar to raw mature areca nut can be manufactured, which are much less in, copper content. The maximum permissible copper content for areca nut products has to be evaluated and curtailed by further research and standardization. Further studies with areca nuts from different locations and more commercial products are recommended. Alkaloids in areca nut which is also believed to play important role in the pathogenesis of OSMF, has to be quantified and compared in raw areca nuts and commercial products.

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