Tannins in Betel Nut and Its Products Consumed in India: Comparison with High-Tannin Sorghum

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The potential carcinogenic properties of the widely consumed betel nut have been illuminated, but other possible harmful effects of this material have received little attention. Here we show that not only betel nut but also other materials popularly consumed with it in betel quid contain very high levels of condensed tannins, which could cause nutritional problems, especially for people whose diets are only marginally adequate.

INTRODUCTION

Betel nut (*Areca catechu* [L.]), also known as areca nut, is an important component of the daily diet of up to $^1/_{10}$ of the world's human population, mainly in Southeast Asia and the South Pacific (Duke, 1985). Betel nut is used most often as a breath-sweetening masticatory, and its consumption is intimately associated with social, cultural, and religious systems of all social classes, rich and poor, in this part of the world. In contrast, high-tannin sorghum is a major dietary staple for the people of the highlands of East Africa and to a lesser extent elsewhere in the world, especially as livestock feed, where birds and other pests limit production of sorghums free of tannin.

In India, betel nut is consumed as whole nuts or as broken pellets. Nuts are usually processed by soaking or boiling with various plant extracts (Raghavan and Baruah, 1958) to reduce the astringency, although raw dried nuts are also consumed in several regions. In most markets in India, betel nuts are available in small pouches in several forms: raw nuts, processed nuts, or "nut powder", which is a combination of betel nut pellets with one or more spices and condiments.

Perhaps the most popular mode of consumption is as "pan", the major ingredients of which are betel nuts and lime paste folded together in betel (*Piper betel* [L.]) leaves. Often one or more other materials such as tobacco, catechu, cloves, cardamon, anise seed, grated coconut, sugar, and other spices or condiments are added. Pan is also referred to in the literature as "betel quid" because it is slowly masticated in the mouth. Some individuals chew as many as 50 quids per day and many sleep with a quid under the tongue (Stich et al., 1982). In some populations the spent quid is expectorated; in others it is swallowed. Betel nut consumption has been occasionally discouraged with little effect (Raghvan and Baruah, 1958), and addiction to betel quid is no less widespread than addiction to alcohol or smoking tobacco. Advertising for "Pan Masala", a ready-for-consumption form packed in small convenient sachets, is targeted for upscale consumers. Pan Masala is now exported from India to countries in the Middle and Far East (Shenolikar, 1989).

The widespread use of these materials raises important questions about the nutritional and health consequences of their consumption, particularly in populations with inadequate diets. The composition of betel nut as summarized by Raghavan and Baruah (1958) includes arecoline and other alkaloids, which are responsible for the stimulating effect on the central nervous system (Duke, 1985), as well as up to 26% tannins including 18% gallotannic acid. In addition to hydrolyzable tannins, betel nuts have been reported to contain condensed tannins (proanthocyanidins) and structurally related monomeric and oligomeric flavan-3-ols (Nonaka et al., 1981; Stich et al., 1983). Other common components of the quid, including betel leaves (Duke, 1985), catechu (katha, an exudate from *Acacia catechu*) (Giri et al., 1987), and some Indian spices and condiments (Narasinga Rao and Prabhavathi, 1982; Nonaka et al., 1983), have been reported to contain high levels of tannin.

The most serious health problem associated with betel nut consumption is oral cancer. The possible role of the tannins of betel quid in causing several hundred thousand oral cancer deaths each year in Asia has been addressed by Stich and his colleagues (Stich et al., 1982, 1983, 1984), Gothoskar and Pai (1986), and Giri et al (1987). In addition to their possible role in carcinogenesis, tannins from sorghum and other foodstuffs have been associated with many antinutritional effects from diminished palatability to inhibition of postdigestive metabolism. These harmful nutritional consequences of tannin consumption have recently been summarized by Salunkhe et al. (1990), who cataloged many dietary sources of tannin including sorghum without addressing the problem of tannins in betel nuts and betel quid.

We have contributed new perspectives to an understanding of the effects of dietary tannins on animal nutrition (Butler, 1989a,b) and of defense mechanisms by which tannin-consuming animals minimize these harmful effects (Mehansho et al., 1987). In the present investigation we have applied some of our methods of tannin analysis to betel nut and betel quid preparations to obtain more complete information on the tannin levels of these products and to compare them with high-tannin sorghum.

METHODS

Popular types of betel nuts, betel nut powders, catechu, and betel quid were procured from the market of Hyderabad, India. Samples were dried at 80 °C and sent to Purdue University for analysis. Sorghum grain was produced at the Purdue University Agronomy Farm under the direction of Dr. John Axtell.

Polyphenol Analyses. Dry samples were ground to a fine powder, and 0.5-g samples were extracted successively with 10 mL each of diethyl ether, methanol (twice), and acidic methanol (1% v/v concentrated HCl in methanol) (twice). After each 20-min extraction (Price et al., 1978), the residue was recovered by centrifugation for extraction with the next solvent. The diethyl ether extract (containing lipid) was discarded. The two methanol extracts were combined for analysis, as were the acidic methanol extracts.

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Table I. Polyphenol Analyses of Betel Quid and Components

sample	extraction solvent[a]	total phenols[b]	flavan-4-ols[c]	proanthocyanidins[c]	flavan-3-ol end[d]	chain length[e]
betel nut varieties						
1. Vakkalu[f]						
nuts	M	200	5.8	41	284	1.0
	H+	221	4.8	45	273	1.0
	total	421	10.6	86	557	
powder	M	255	9.7	62	346	1.0
	H+	209	5.2	49	255	1.0
	total	464	14.9	111	601	
2. Chikni[g] (unpolished)						
nuts	M	216	15.6	144	355	2.2
	H+	231	6.1	154	373	2.5
	total	447	21.7	298	728	
powder	M	236	50.7	148	360	2.6
	H+	253	6.7	161	382	2.6
	total	489	57.4	309	742	
3. Chikni[h] (polished)						
nuts	M	137	9.2	159	378	2.6
	H+	219	14.0	146	343	2.6
	total	356	23.2	305	721	
powder	M	229	14.8	159	361	2.7
	H+	248	4.8	159	356	2.7
	total	477	19.6	318	717	
4. Chalia[i] (raw)						
nuts	M	200	0.3	22	37	3.3
	H+	247	21.1	151	334	2.8
	total	447	21.4	173	371	
powder	M	232	0.4	26	38	3.6
	H+	253	21.5	156	330	2.0
	total	485	21.9	182	368	
5. Chalia (roasted)						
nuts	M	218	1.5	33	78	2.6
	H+	253	16.7	142	326	2.6
	total	471	18.2	175	404	
powder	M	222	1.2	36	54	3.6
	H+	252	17.6	156	360	2.6
	total	474	18.8	192	414	
6. Triveni[j]						
nuts	M	137	6.2	75	171	2.7
	H+	197	6.3	86	202	2.6
	total	334	12.5	161	373	
powder	M	199	8.3	111	259	2.6
	H+	173	2.2	81	192	2.6
	total	372	10.5	192	451	
7. Crane[j]						
nuts	M	261	14.1	158	333	2.8
	H+	230	7.8	118	277	2.6
	total	491	21.9	276	610	
powder	M	219	14.0	158	364	2.5
	H+	224	7.8	115	256	2.7
	total	443	21.8	273	620	
Catechu (Katha)						
8. sample A[k]	M	376	4.7	38	382	1.0
	H+	58	0.4	2	11	1.0
	total	434	5.1	40	426	
9. sample B[l]	M	380	4.8	79	385	1.8
	H+	106	0.6	4	86	1.3
	total	486	5.4	83	471	
betel quid						
10. Saada[m]	M	110	10.5	88	149	3.2
	H+	73	2.5	56	52	5.0
	total	183	13.0	144	201	
11. Meetha[n]	M	67	4.4	51	69	3.5
	H+	68	2.0	29	49	4.2
	total	135	6.4	80	118	
12. Zarda[o]	M	122	8.9	88	157	3.1
	H+	90	1.8	46	36	5.9
	total	212	10.7	134	193	
13. Sauf Copra[p]	M	59	5.6	53	84	3.7
	H+	47	1.5	31	31	5.0
	total	106	7.1	84	115	
14. Ram Pyari[q]	M	113	9.6	107	118	4.4
	H+	87	2.9	48	74	3.5
	total	200	12.5	155	192	

Table I. (Continued)

sample	extraction solvent[a]	total phenols[b]	flavan-4-ols[c]	proanthocyanidins[c]	flavan-3-ol end[d]	chain length[e]
sorghum						
15. ISO469 (tannin-free)						
	M	1.3	ND	ND	0.1	
	H+	2.3	ND	ND	ND	
	total	3.6			0.1	
16. BR64 (high tannin)						
	M	37.6	3.1	20	59	2.3
	H+	2.3	0.2	5	11	2.8
	total	39.9	3.3	25	70	

[a] M = methanol; H+ = acidic methanol (see Methods). [b] Prussian blue assay, A720/g (Price and Butler, 1977). [c] Flavan-4-ol and proanthocyanidin assay, A550/g (Watterson and Butler, 1983). [d] Flavan-3-ol end groups by the vanillin assay, A510/g (Butler et al., 1982). [e] Chain length estimate (Putman and Butler, 1985). [f] From Shimoga, Karnatana state; processed by boiling and drying. [g] From Assam state. [h] Polished with catechu. [i] From Bombay, unprocessed. [j] Commercial product popular in Andhra Pradesh. [k] Several samples obtained from the Hyderabad market gave similar analyses. [l] One of several samples imported from India by Bombay Bazar, 548 Valencia St, San Francisco, all of which gave similar analyses. [m] In addition to the basic ingredients (betel nut, betal leaf, and lime) common to all the betel quids analyzed, Saada (plain) contained catechu, anise seed, and mint. [n] Additional ingredients: catechu, anise seed, mint, rose petal preserve, and grated coconut. [o] (Golden Kashmiri) Additional ingredients: catechu and tobacco extract. [p] Additional ingredients: catechu and grated coconut. [q] Additional ingredients: catechu, cardamon seeds, and special spices. [r] None detected.

Each of the combined extracts was separately analyzed for total phenols (spectrophotometric Prussian blue assay; Price and Butler, 1977), flavan-4-ols and proanthocyanidins (Watterson and Butler, 1983) without the poly(vinylpyrrolidone), and condensed tannins (vanillin assay in glacial acetic acid; Butler et al., 1982). Average relative chain length of the condensed tannins in the extracts was calculated as described by Putman and Butler (1985). At the time these analyses were done, the new specific assay for hydrolyzable tannins (Inoue and Hagerman, 1988) was not available.

RESULTS AND DISCUSSION

The results of our polyphenol assays on betel nut preparations, associated products, and typical high- and low-tannin sorghums are presented in Table I. Because tannin purified from each of the materials analyzed was not available for use as an appropriate standard (Hagerman and Butler, 1989) and because no single sample of pure tannin could adequately serve as standard for the various tannins in these complex mixtures of materials, the data are presented as absorbance units per gram rather than as percent composition, which would be misleading. Because the residue of the methanol (M) extractions was re-extracted twice with acidic methanol (H+) and the resulting combined extract analyzed separately from the combined methanol extracts, the value for the total extractable material is the sum of the values for the two combined extracts of each sample. Although moisture, which can interfere with tannin extraction (Butler, 1982), was removed by drying, it is likely that not all the tannin present was extractable, so these values represent the lower limit of what was actually present.

In contrast to sorghum, in which tannin is largely extractable in methanol (or in acidic methanol in group II sorghums; Price et al., 1978), most of the betel nut preparations yielded approximately equal amounts of tannin in the methanol and the subsequent acidic methanol extracts. Chalia nut preparations are like group II sorghums in that most of the tannin is extractable only in acidic methanol; this characteristic is not affected by roasting. In contrast, in catechu the tannin is largely extractable in methanol, and the similar result with betel quids may reflect their catechu content.

Flavan-4-ols (luteoforol and apiforol in sorghum) are monomeric flavonoids rather than polymeric condensed tannins. Flavan-4-ols in sorghum appear to be precursors of the 3-deoxyanthocyanidin pigments luteolinidin and apigeninidin, which are alternate end products, with condensed tannins, of flavonoid metabolism (Watterson and Butler, 1983). Their only reported physiological activity is contribution to mold resistance in sorghum (Jambunathan et al., 1990). Flavan-4-ols occur independently of tannin in sorghum, and, like tannin, the level that is present varies greatly for different cultivars; a similar variability between betel nut preparations may account for the differences observed in flavan-4-ol content.

Unless the components in these materials produce absorbance values in these assays greatly different from the sorghum tannin we have previously purified and characterized (Hagerman and Butler, 1980; Asquith et al., 1983), these results indicate that betel nuts and materials consumed with them contain condensed tannins (proanthocyanidins) at levels considerably higher than that of any other foodstuff we have analyzed or have seen reported (Price and Butler, 1980). BR64, a typical high-tannin bird-resistant sorghum, contains about 2.5% extractable tannin. When compared to tannin-free sorghums, diets based on this and similar sorghums severely limit weight gains of experimental animals such as rats (Price et al., 1980) and chicks (Price et al., 1979) and cause numerous abnormalities in chick leg development (Price et al., 1979). Dietary tannin at this level can therefore have severe nutritional consequences.

Assuming betel nut and associated tannins give absorbance values per gram similar to those of sorghum tannin, these absorbance values can be used to estimate percent tannin content. On this basis, chikni nut powder (the most tannin rich of the samples analyzed) contained about 31% tannin by the proanthocyanidin assay, about 27% tannin by vanillin assay for flavan-3-ol end groups, and about 33% tannin by the Prussian blue assay for total phenols. Vakkalu nuts contained the lowest levels of tannin: 8.7% (proanthocyanidin assay), 20% (vanillin assay), and 29% (total phenols). Values for catechu were similar to those for Vakkalu nuts: 4–8% tannin (proanthocyanidin), 20% (vanillin), and 29% (total phenols). Differences in these values reflect differences in proportions and responsiveness of various phenolic components to these assays and reinforce our suggestion that no single polyphenol/tannin assay is appropriate for all samples (Hagerman and Butler, 1989). Estimates of relative chain length of condensed tannins gave similar values for betel nut and sorghum tannins.

Betel quids contained somewhat lower levels of tannin, reflecting the presence of tannin-free ingredients such as lime. Again assuming similarity to sorghum tannin, the most tannin rich quid was Ram Pyari, with 15.5% (proanthocyanidin assay), 6.9% (vanillin assay), and 13.8% (total phenol assay). The lowest levels of tannin, a little over half of the above, were found in Sauf Copra quid. While these tannin levels are considerably less than those found

in betel nuts and powder, they are nevertheless several-fold higher than the levels found in high-tannin sorghum.

Traditional methods of processing high-tannin sorghum include soaking with ash or other alkaline materials, which largely eliminates assayable tannin and its associated antinutritional effects (Price et al., 1979; Muindi and Thomke, 1981). It is possible that when betel nuts are consumed with lime as in betel quid, the tannins are similarly "detoxified" by the alkaline conditions. In the dry quids we analyzed, we found no evidence of diminished extractability/assayability due to the presence of lime. In some cases we assayed for protein-precipitable phenols (Hagerman and Butler, 1978) and found that betel nut and betel quid tannins are somewhat less effectively precipitated by protein than are sorghum tannins. The average ratio of protein-precipitable phenols to proanthocyanidins in pan was 60% of the value for the nut powders, suggesting little influence of the lime in the dry sample. When the quids are moistened during chewing, however, it is likely that the lime further diminishes the toxicity of the betel quid tannins. Consumption of betel nuts without lime (because of convenience and lower cost, this is the predominant form of consumption) would seem to result in the most serious nutritional consequences. The magnitude of the problem may be much greater in populations such as rural India, where the staple grains like sorghum or other cereals provide most of the protein requirement and nitrogen balance may be precarious due to marginal quality of these proteins. Fortunately, Indian sorghums are relatively low in tannin (Radhakrishnan and Sivaprasad, 1980).

A unique group of salivary proteins that contain high levels (up to 45%) of proline account for about 70% of the protein in human saliva. These proteins have an extraordinarily high affinity for dietary tannin and seem to moderate its otherwise much more harmful effects (Mehansho et al., 1987). Addition of tannin to rat diets induces production of these proline-rich tannin-binding proteins, which may account for the associated increase in fecal protein and proline/protein ratio (Butler, 1989b). Although binding and loss of dietary proteins may be spared by these endogenous proteins which contain very low levels of indispensable amino acids, their loss to the feces as a complex with tannin or their loss by expectoration nevertheless represents a net loss of body protein. Rats fed propanolol, a common heart drug, cannot make these salivary tannin-binding proteins, and the antinutritional effects of dietary tannin are much more severe in these animals (Butler, 1989b). If similar effects occur in humans, betel nut chewers being treated with propanolol for a heart condition could be significantly increasing their vulnerability to the antinutritional effects of betel tannins.

Consumption of betel quid (pan) is not effectively discouraged in India. On the contrary, it is popularly believed to supply nutrients such as vitamin A and calcium because it contains betel leaves and lime. Though efforts are being made to discourage people from choosing tobacco or betel quid containing tobacco in view of tobacco's apparent carcinogenic effects, the immediate nutritional damage that can be caused by chewing betel nut powder has not received adequate emphasis. Consumption of such tannin-rich products by populations where the nutritional status is poor is counterproductive to the efforts of breeders and nutritionists attempting to reduce the tannin content of natural foods and thereby improve the nutritional value of the diet.

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