Contents of Phenolics and Alkaloids in *Areca catechu* Linn. during Maturation

Chin-Kun Wang,* Wen-Hsiu Lee, and Chin-Hui Peng

Graduate Institute of Nutritional Science, Chung Shan Medical and Dental College, 113, Section 2, Ta-Chien Street, Taichung, Taiwan, Republic of China

Betel quid is a popular masticatory in Taiwan. Fresh unripe areca fruit, the fruit of *Areca catechu* Linn., is the main ingredient of betel quid. In this study, the information on the measurements of phenolics and alkaloids in *A. catechu* Linn. was obtained. The phenolics in *A. catechu* Linn. were mainly distributed in root followed by fresh unripe fruit, leaf, spike, and vein, while the contents of alkaloids in *A. catechu* Linn. were in the order of root, fresh unripe fruit, spike, leaf, and vein. Total amounts of phenolics in areca fruit were well correlated with the length and maturation, but those of alkaloids were only correlated with the maturation. Upside-down areca fruit, areca fruit growing upward (opposite to normal fruits, growing downward), contained a much higher amount of arecaidine (4 mg/g of fresh wt) than normal fresh unripe areca fruit (1.5 mg/g of fresh wt). Tender shoot, the upper young stem of the tree, cooked as a delicious syrup, contained a small amount of total phenolics (0.58 mg of gallic acid equiv/g of fresh wt), condensed tannin (0.85 mg of catechin equiv/g of fresh wt), and total alkaloids (2.38 mg/g of fresh wt).

Keywords: *Areca catechu Linn.; phenolics; alkaloids; areca fruit; maturation; upside-down areca fruit; tender shoot*

INTRODUCTION

Being the most popular masticatory in Taiwan, betel quid is primarily composed of fresh areca fruit, *Piper betle* (inflorescence or leaf), and lime paste (Ko et al., 1992). Many people have the hobby of chewing betel quid due to its physiological effects, including increasing stamina and general well-being (Nieschulz, 1967; Hwang et al., 1992).

Fresh areca fruit, the fruit of *Areca catechu* Linn., contains some physiologically active substances (e.g., phenolics and alkaloids). The phenolics in areca fruit involve condensed tannins, hydrolyzable tannins, nontannin flavans (e.g., catechin, epicatechin), and simple phenolics (Wang and Lee, 1996; Wang and Hwang, 1993a). The crude phenolic extract of areca fruit and its two separated fractions (condensed and noncondensed tannin phenol fractions) exhibit marked antioxidative activity and an antimutagenic effect on 2-amino-3-methylimidazo[4,5-*f*]quinoline toward *Salmonella typhimurium* TA98 and TA100 (Wang and Lee, 1996). Catechin has been shown to be antimutagenic against environmental mutagens (Nagabhushan and Bhide, 1988). Aqueous extract, catechin extract, and tannin extract of areca nut are shown to depress the formation of *N*-nitroso-L-proline in the human body (Stich et al., 1983). However, the catechin and tannin extracts of areca nut inhibit the activity of collagenase (Scutt et al., 1987) and stimulate the synthesis of collagen (Canniff and Harvey, 1981). It seems that phenolics of areca nut are closely related to oral submucous fibrosis.

The areca fruits have been shown to contain four related pyridine alkaloids: arecoline, arecaidine, guvacoline, and guvacine (Wang and Hwang, 1993b). Arecoline and govacoline are converted into arecaidine and guvacine under alkaline conditions, respectively (Wang and Hwang, 1993b). Arecoline, the major alkaloid in areca fruit, as an agonist for the muscarinic receptor, has a stimulating parasympathetic action and cardiovascular and ocular effects (Mujumdar et al., 1979; Taylor, 1990). Intravenous administration of arecoline causes elevations of heart rate and blood pressure (Janowsky and Risch, 1984). Guvacine and arecaidine are potent inhibitors of the uptake of the central inhibitory transmitter γ-aminobutyric acid (GABA) (Johnston et al., 1975).

Areca nut, one component of betel quid used in India and other Southern Asian areas, is obtained from the fruit of the *Areca catechu* tree. The outer pericarp of the ripe fruit, which is orange-yellow, is removed to separate the nut (Bhosle et al., 1992). In Taiwan, fresh unripe areca fruit (not areca nut) is the main ingredient of betel quid. Except for areca fruit, tender shoot (upper young stem of *A. catechu* tree) is edible and cooked as a syrup. Upside-down areca fruit, areca fruit growing upward (opposite to the other fruits, growing downward), can not be distinguished from normal fruits by appearance after harvesting. Zero, one, or two upside-down fruits grow in a *A. catechu* tree. Chewing of betel quid containing upside-down areca fruit is said to induce strong physiological responses, even death.

The changes of phenolics in betel nut during maturation are discussed (Mathew and Govindarajan, 1964). However, the contents of phenolics and alkaloids in *A. catechu* Linn. (fresh areca fruits of various maturities, upside-down areca fruit, tender shoot, root, leaf, spike, and vein of the tree) were still deficient. The object of this study was to obtain more complete information on the contents of phenolics and alkaloids in *A. catechu* Linn.

MATERIALS AND METHODS

Materials. Various maturities of areca fruits, upside-down areca fruit, tender shoot, root, leaf, spike, and vein of *A. catechu* tree (3−4 m in height) were obtained from a farm in

* Author to whom correspondence should be addressed (telephone, 886-4-3846164; fax, 886-4-3890964; e-mail, wck@mercury.csmc.edu.tw).

S0021-8561(96)00547-X CCC: $14.00

Nantou County, Taiwan. They were either used immediately or stored at 4 °C before use. Cinchonine sulfate and standard phenolic compounds (catechin, gallic acid) and alkaloids (arecoline, guvacine) were the products of Sigma Chemical Co. (St. Louis, MO). Guvacoline and guvacine were obtained by the method in our previous work (Wang and Hwang, 1993b).

Extraction of Phenolics. Phenolic extracts of all test samples were prepared according to the method described in our previous work (Wang and Hwang, 1993a). In brief, a sample of 8 g was ground with 80% aqueous acetone in a Waring blender and extracted for 1 h three times with 30 mL portions, at 25 °C in the dark. The final volume was adjusted to 100 mL with 80% aqueous acetone. The contents of phenolics were determined from the phenolic extract solution (100 mL).

Total Phenolic Determination. Phenolic extract solution (50 μL) was diluted with water to 2 mL in a 10 mL measuring flask; 1 mL of Folin−Ciocalteu phenol reagent was added and the flask vigorously shaken. Immediately, 5 mL of 20% sodium carbonate was pipetted and the mixture made up to 10 mL, shaking thoroughly again. After 20 min, the absorbance of the mixture was read at 735 nm by using a spectrophotometer (U-3000, Hitachi, Japan) (Julkunen-Tiitto, 1985). The results were plotted after a gallic acid standard made in the same manner. The contents of total phenolics are expressed as mg of gallic acid equiv/g of sample.

Condensed Tannin Determination with Vanillin−HCl. Phenolic extract solution (0.1 mL) was taken and put into tubes covered with aluminum foil; 3 mL of 4% vanillin (w/v) in methanol was added, and the tubes were shaken vigorously with a mixture. Immediately after that 1.5 mL of concentrated HCl was pipetted, and the tubes were shaken again. The absorbance was read at 500 nm after the mixture was allowed to stand for 20 min at room temperature (Julkunen-Tiitto, 1985). The results were plotted after a catechin standard made in the same manner. The contents of condensed tannin are expressed as mg of catechin equiv/g of sample.

Estimation of Different Phenolic Groups. The contents of simple phenolics, non-tannin flavans, and hydrolyzable and condensed tannins in the phenolic extracts were determined by the method of Peri and Pompei (1971) and are expressed as mg of catechin equiv/g of sample.

Extraction and Analysis of Alkaloids. The extracts of alkaloids were prepared according to the method described in our previous work (Wang and Hwang, 1993b). In brief, each sample (100 g) was extracted with 0.001 M orthophosphoric acid (500 mL). The acidic and neutral components in the aqueous extract were removed by extracting with chloroform. The contents of alkaloids in the aqueous residue were determined by the method of HPLC as described by Huang and McLeish (1989).

Statistical Analysis. Results in this paper were evaluated by the analysis of variance (ANOVA) followed by Duncan's new multiple range test to separate the means. Statistics were performed by using the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Measurements of Phenolics. As illustrated in Table 1, the contents of total phenolics and condensed tannin were very rich in root (17.14 and 18.05 mg/g of fresh wt) followed by areca fruit, leaf, spike, vein, and tender shoot. Edible tender shoot contained few total phenolics and condensed tannin (0.58 and 0.85 mg/g of fresh wt), which might explain why the syrup of tender shoot tasted nonastringent. For the measurement of phenolics in areca fruit during maturation, five maturities of areca fruits (calyx, flower, two unripe areca fruits, and ripe areca fruit) were collected. Results showed that the contents of total phenolics and condensed tannin increased in a maturity dependent manner (Table 1). Such correlation is the same as that reported by Mathew and Govindarajan (1964).

Table 1. Contents of Total Phenolics and Condensed Tannins in *A. catechu* Linn.

sample	content (mg/g of fresh wt)	
	total phenolics[a]	condensed tannin[b]
root	17.14 ± 0.33[a,c]	18.05 ± 6.61[a]
leaf	5.49 ± 0.36[d]	3.67 ± 0.66[c]
spike	4.72 ± 0.90[d]	1.78 ± 0.47[e]
vein	2.41 ± 1.31[e]	1.33 ± 1.03[e]
tender shoot	0.58 ± 0.01[f]	0.85 ± 0.16[e]
calyx	3.52 ± 0.51[d]	1.22 ± 0.36[e]
flower	3.83 ± 0.81[d]	2.15 ± 0.45[d]
unripe fruit (2 cm)[d]	5.78 ± 0.86[d]	9.03 ± 1.90[b]
unripe fruit (3 cm)[e]	9.28 ± 0.65[c]	7.84 ± 0.95[b]
ripe fruit	12.63 ± 0.41[b]	9.85 ± 0.88[b]
upside-down fruit	8.79 ± 0.32[c]	8.32 ± 0.41[b]

[a] Units: mg of gallic acid equiv/g of fresh wt. [b] Units: mg of cateching equiv/g of fresh wt. [c] Data bearing different superscript letters in the same column are significantly different ($p < 0.05$). [d] Unripe areca fruit of 2 cm in length. [e] Unripe areca fruit of 3 cm in length (commercial size).

An opposite growing direction is the main difference between upside-down areca fruit and normal areca fruit. The measurement of phenolics showed that the contents of phenolics in unripe areca fruit (commercial type) were closely similar to those of upside-down areca fruit. To further understand the detailed difference of phenolics between commercial unripe areca fruit and upside-down areca fruit, a well-differentiated distribution picture was obtained by applying the Folin−Ciocalteu method to the phenolic groups resulting from combined precipitations with both formaldehyde and cinchonine (Peri and Pompei, 1971). According to this procedure, the "total phenolics" value is from the sum of four groups of phenolic compounds: Condensed tannins were the polymers or copolymers of catechin and leucoanthocyanins. Hydrolyzable tannins were polyesters of a sugar or related polyhydric alcohols and a phenolic carboxylic acid, usually gallic or ellagic acid. Non-tannin flavans included monomeric anthocyanins, catechins, and leucoanthocyanins. Simple phenolics were the derivatives of hydroxybenzoic and hydroxycinnamic acids. Results also showed no significance between these two fruits by comparing the four phenolic groups (Figure 1).

For the different species, maturity, size, and shape of commercial areca fruit used in Taiwan, it is necessary to summerize the contents of phenolics in unripe green areca fruits. Figure 2 clearly indicated that the contents of total phenolics and condensed tannin were closely related to the length of fresh areca fruit. The intake of phenolics of areca fruit could be well estimated by the consumption of areca fruit (the amount and length of areca fruit). Further studies about the role of phenolics in the toxicity or oral diseases caused by betel quid chewing could be discussed according to this data.

Measurements of Alkaloids. As illustrated in Table 2, the total amount of four alkaloids (arecoline, arecaidine, guvacoline, and guvacine) of *A. catechu* Linn. were in the order of root (25.54 mg/g of fresh wt), areca fruit (13.63 mg/g of fresh wt), spike (12.02 mg/g of fresh wt), tender shoot (2.38 mg/g of fresh wt), leaf (1.46 mg/g of fresh wt), and vein (0.9 mg/g of fresh wt). The root contained high levels of arecaidine (13.72 mg/g of fresh wt) and guvacine (12.92 mg/g of fresh wt). The spike contained arecaidine, guvacoline, guvacine, and one unknown substance (at retention time of 6.5 min); arecaidine (8.55 mg/g of fresh wt) was the most abundant one. The areca fruit involved the four alkaloids; arecoline was the major one (7.52 mg/g of fresh wt), and arecaidine was the least one (1.51 mg/g of fresh wt).

Table 2. Contents of Alkaloids in *A. catechu* Linn.

sample	content (mg/g of fresh wt)				
	arecoline	arecaidine	guvacoline	guvacine	total
root	3.29 ± 1.13[b] [a]	13.72 ± 0.63[a]	0.50 ± 0.01[b]	12.92 ± 1.61[a]	25.54 ± 2.07[a]
spike	nd[b]	8.55 ± 1.20[b]	0.09 ± 0.01[d]	2.68 ± 0.65[b]	12.02 ± 1.36[b]
tender shoot	0.29 ± 0.03[e]	1.75 ± 0.06[d]	nd	0.34 ± 0.01[d]	2.38 ± 0.07[d]
leaf	0.42 ± 0.26[e]	0.20 ± 0.07[e]	0.31 ± 0.08[c]	0.38 ± 0.07[d]	1.46 ± 0.29[e]
vein	0.22 ± 0.08[e]	0.22 ± 0.05[e]	0.21 ± 0.02[c]	0.27 ± 0.09[d]	0.90 ± 0.13[f]
calyx	1.82 ± 0.18[c]	1.22 ± 0.15[d]	0.23 ± 0.06[c]	0.62 ± 0.11[c]	3.89 ± 0.18[c]
flower	1.25 ± 0.19[d]	1.44 ± 0.23[d]	0.42 ± 0.08[b]	0.57 ± 0.07[c]	3.68 ± 0.15[c]
unripe fruit (2 cm)[c]	7.52 ± 0.27[a]	1.51 ± 0.18[d]	2.02 ± 0.20[a]	2.58 ± 0.39[b]	13.63 ± 0.25[b]
areca fruit (3 cm)[d]	7.76 ± 0.47[a]	1.53 ± 0.34[d]	2.25 ± 0.12[a]	2.76 ± 0.15[b]	14.35 ± 0.38[b]
ripe fruit	1.55 ± 0.09[d]	nd	nd	nd	nd
upside-down fruit	8.05 ± 0.34[a]	4.15 ± 0.48[c]	1.25 ± 0.32[b]	0.47 ± 0.09[c]	13.92 ± 0.43[b]

[a] Data bearing different superscript letters in the same column are significantly different ($p < 0.05$). [b] nd, no data. [c] Unripe areca fruit of 2 cm in length. [d] Unripe areca fruit of 3 cm in length (commercial size).

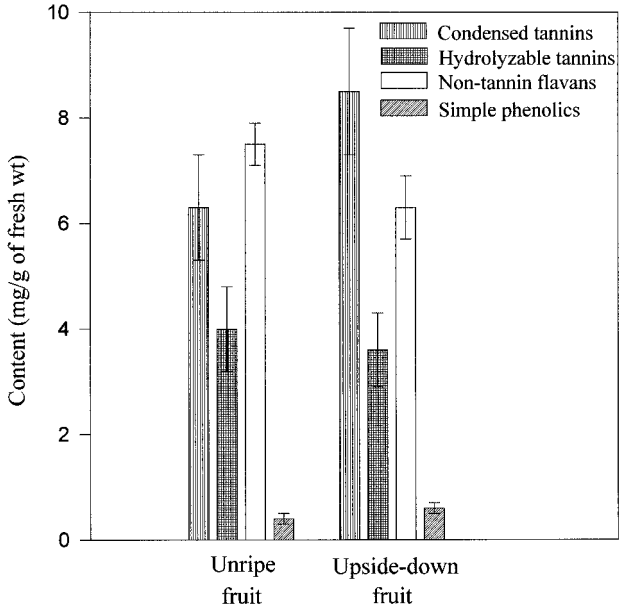

Figure 1. Contents of condensed tannins, hydrolyzable tannins, non-tannin flavans, and simple phenolics in areca fruit and upside-down areca fruit.

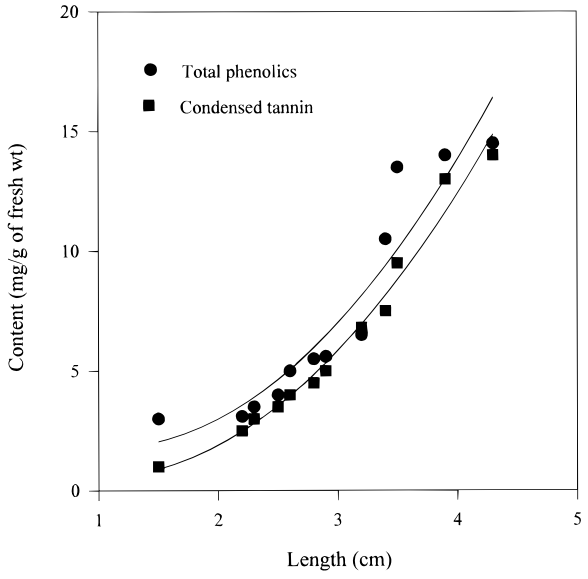

Figure 2. Contents of phenolics in various lengths of areca fruits. Total phenolics: $y = 1.137x^2 - 2.407x + 4.077$, $r = 0.827$. Condensed tannin: $y = 1.795x^2 - 3.088x + 1.85$, $r = 0.924$.

There are about 300−400 areca fruits growing in one spike. Arecoline is the methyl ester of arecaidine (Manske and Holmes, 1950), and the conversion between arecaidine and arecoline via the junction of areca fruit and spike may explain such a distribution. Tender shoot contained a little arecaidine (1.75 mg/g of fresh wt), no guvacoline, a little arecoline, and guvacine. Tender shoot gives a very weak physiological responses that could be due to the low levels of alkaloids. The measurements of alkaloids in areca fruits (the same five samples as described above) during maturation also showed an increase in a maturity dependent manner. The contents peaked for all four alkaloids in commercial type (unripe green areca fruit), but the ripe areca fruit contained only some arecoline (Table 2).

For the strong physiological responses of chewing betel quid containing upside-down areca fruit and the marked pharmacological effects of alkaloids from areca fruit (Mujumdar et al., 1979), the contents of alkaloids between unripe areca fruit and upside-down areca fruit were compared. It revealed that the content of arecaidine in upside-down areca fruit was 2 times more than that of unripe areca fruit (commercial type). Arecaidine is a potent inhibitor of the uptake of the central inhibitory transmitter GABA (Johnston et al., 1975) and induces much secretion of catecholamine from adrenal chromaffin cells (Hwang et al., 1993). This was perhaps

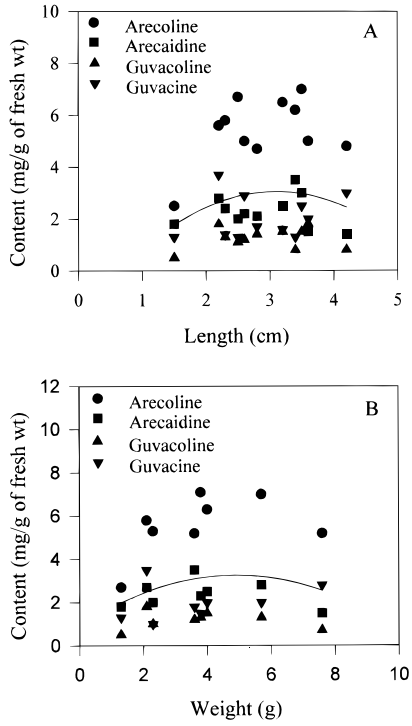

Figure 3. Contents of alkaloids in various lengths (A) and weights (B) of areca fruits.

the reason why people said that betel quid containing upside-down areca fruit induced strong physiological responses during the chewing session. To summarize the contents of alkaloids in unripe areca fruit, Figure 3 shows that the content of arecoline and total amounts of alkaloids in commercial areca fruit increased slightly with the length (1.5−3.3 cm) and weight (1−5 g) but decreased at longer size (>3.3 cm) and larger weight (>5 g).

Conclusions. Our results indicate clearly that the root of *A. catechu* Linn. involves the most abundant phenolics and alkaloids. Total amounts of phenolics in areca fruit are well correlated with the length and maturation, but those of alkaloids were only with the maturation. The content of arecaidine in upside-down areca fruit is 2 times more than that found in normal areca fruit. It seems that little phenolics and alkaloids in tender shoot lead to the syrup of tender shoot tasting nonastringent and to nonstimulating responses. This work also notes that the intake of alkaloids and phenolics can be well estimated by the maturation and size. Further research on the role of alkaloids and phenolics in the oral diseases caused by betel quid chewing is required according to these data.

LITERATURE CITED

Bhosle, R. B.; Murti, P. R.; Gupta, P. C. Tobacco habits in India. In *The control of tobacco-related cancers and other disease*; Prakash, C., Hamner, J. E., Murti, P. R., Eds.; Oxford University Press: Bombay, 1992; pp 25−46.

Canniff, J. P.; Harvey, W. The aetiology of oral submucous fibrosis: the stimulation of collagen synthesis by extracts of areca nut. *Int. J. Oral Surg.* **1981**, *10*, 163−167.

Huang, J. L.; McLeish, M. J. High performance liquid chromatographic determination of the alkaloids in betel nut. *J. Chromatogr.* **1989**, *475*, 447−450.

Hwang, L. S.; Wang, C.-K.; Sheu, M.-J.; Kao, L.-S. Phenolic compounds of *Piper betle* flower as flavoring and neuronal modulating agents. In *Phenolic Compounds in Food and Their Effects on Health I*; Ho, C. T., Lee, C. Y., Huang, M. T., Eds.; ACS Symposium Series 506; American Chemical Society: Washington, DC, 1992; pp 200−213.

Hwang, L. S.; Wang, C.-K.; Kao, L.-S. Neuronal activity modulating components in betel quid. Symposium on betel quid chewing and its health effect, Kaohsiung, Taiwan, 1993.

Janowsky, D. S.; Risch, S. C. Cholinomimetic and anticholinergic drugs used to investigate an acetylcholine hypothesis of affective disorders and stress. *Drug. Dev. Res.* **1984**, *4*, 125−129.

Johnston, G. A. R.; Krogsgaard-Larsen, P.; Stephanson, A. Betel nut constituents as inhibitors of γ-aminobutyric acid uptake. *Nature* **1975**, *258*, 627−628.

Julkunen-Tiitto, R. Phenolic constituents in the leaves of Northern willows: Methods for the analysis of certain phenolics. *J. Agric. Food Chem.* **1985**, *33*, 213−217.

Ko, Y. C.; Chiang, T. A.; Chang, S. J.; Hsieh, S. F. Prevalence of betel quid chewing habit in Taiwan and related sociodemographic factors. *J. Oral Pathol. Med.* **1992**, *21*, 261−264.

Manske, R. H. F.; Holmes, H. L. *The alkaloids Vol. I*; Academic Press: New York, 1950; pp 171−175.

Mathew, A. G.; Govindarajan, V. S. Polyphenolic substances of areca nut II. Changes during maturation and ripening. *Phytochemistry* **1964**, *3*, 657−665.

Mujumdar, A. M.; Kapadi, A. H.; Pendse, G. S. Chemistry and pharmacology of betel nut *Areca catechu* Linn. *J. Plant Crops* **1979**, *7*, 69−92.

Nagabhushan, M.; Bhide, S. V. Antimutagenicity of catechin against environmental mutagens. *Mutagenesis* **1988**, *3*, 293−296.

Nieschulz, O. Pharmacology of the active principle of betel (I) central effects of arecoline. *Arzneim. Forsch.* **1967**, *17*, 1292−1295.

Peri, C.; Pompei, C. Estimation of different phenolic groups in vegetable extracts. *Phytochemistry* **1971**, *10*, 2187−2189.

Scutt, A.; Mehgji, S.; Canniff, J. P.; Harvey, W. Stabilization of collagen by betel nut polyphenols as a mechanism in oral submucous fibrosis. *Experientia* **1987**, *43*, 391−393.

Stich, H. F.; Ohshima, H.; Pignatelli, B.; Michelon, J.; Bartsch, H. Inhibitory effect of betel nut extracts on endogenous nitrosation in humans. *J. Natl. Cancer Inst.* **1983**, *70*, 1047−1050.

Taylor, P. Cholinergic agonists. In *The Pharmacological Basis of Theraperuics*; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990.

Wang, C.-K.; Hwang, L. S. Analysis of the phenolic compounds in betel quid. *J. Chin. Agric. Chem. Soc.* **1993a**, *31*, 623−632.

Wang, C.-K.; Hwang, L. S. Study on the separation and hydrolysis of alkaloids from betel nut. *Food. Sci.* **1993b**, *20*, 514−526.

Wang, C.-K.; Lee, W.-H. The separation, characteristics and biological activities of phenolics in areca fruit. *J. Agric. Food Chem.* **1996**, *44*, 2014−2019.

Received for review July 22, 1996. Revised manuscript received December 2, 1996. Accepted December 13, 1996.[⊗] This research work was supported by the National Science Council, Republic of China, under Grant NSC 83-0412-B040-015 M14.

JF960547Q

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1997.