Inhibitory Effect of Betel Quid on the Volatility of Methyl Mercaptan

Chin-Kun Wang,* Su-Lin Chen, and Mei-Guey Wu

Graduate Institute of Nutritional Science, Chung Shan Medical and Dental College, 110, Section 1, Chien-Kuo North Road, Taichung, Taiwan, Republic of China

Betel quid, a popular natural masticatory in Taiwan, is mainly composed of fresh areca fruit, *Piper betle* (leaf or inflorescence), and slaked lime paste. People say that halitosis disappears during betel quid chewing. In this study, the removal of mouth odor during betel quid chewing was discussed by using a model systemwhich measured its inhibition on the volatility of methyl mercaptan. Results showed that crude extracts of betel quid (the mixture of areca fruit, *Piper betle,* and slaked lime paste) and extracts of the mixture of areca fruit and slaked lime paste exhibited marked effects on the volatility of methyl mercaptan, and the inhibition function increased when increasing amounts of slaked lime paste were added. The same condition (increased inhibition) was also found by replacing the slaked lime paste with alkaline salts (calcium hydroxide, potassium hydroxide, or sodium hydroxide). Areca fruit, the major ingredient of betel quid, contained abundant phenolics. However, the crude phenolic extract of areca fruit did not show any inhibitory activity on the volatility of methyl mercaptan. Great inhibitory activity occurred only when the crude phenolic extract of areca fruit was treated with alkali. Further studies by using gel filtration determined that the effect probably came from the oxidative polymerization of phenolics of areca fruit after alkaline treatment.

Keywords: *Betel quid; halitosis; methyl mercaptan; phenolics; gel filtration*

INTRODUCTION

Betel quid is a natural masticatory, and is very popular in Taiwan (*1*). Epidemiological studies showed that betel quid chewing is closely related to oral diseases (e.g., leukoplakia, or oral submucous fibrosis and oral cancer; *2*, *3*). However, people have the habit of chewing betel quid mainly because of some physiological responses (including increased stamina, a general feeling of well-being, slightly drunk feeling, salivation, cardioacceleration, and removal of halitosis, etc.) during the chewing session (*4*−*6*).

Halitosis is a syndrome of releasing off-odor from the mouth. Oral malodor originates mainly from the bacterial decomposition of proteins in food detritus, skin residue, and oral bacteria (*7, 8*). The substances principally responsible for oral malodor are volatile sulfur or nitrogen compounds, short chain fatty acids, alcohols, and aldehydes. Among these odorous compounds, the major constituents are primarily sulfur compounds such as methyl mercaptan, hydrogen sulfide, and dimethyl sulfide (*7*). Various methods are used to remove halitosis. Oral deodorizers are the most effective and convenient way to accomplish its removal. Some natural plant extracts (e.g., thyme, rosemary, tea, marine algae, and sage, etc.) are found to clear halitosis effectively (*9*−*12*). Chlorophylls and their derivatives have been the most popular commercial natural deodorizers (*13*). Flavonoids are another important group of natural deodorants (*14*−*16*).

Fresh areca fruit, *Piper betle* (inflorescence of leaf), and slaked lime paste are important ingredients of betel quid in Taiwan. Phenolics and alkaloids of these ingredients were found to exhibit special biological activities (*17*−*22*). The neuronal activities of chewing betel quid have been confirmed in our previous works (*5, 23*). The changes and biological activity of physiologically active components of betel quid also have been determined (*21, 22, 24*−*27*). However, the mechanism of removal of halitosis by chewing betel quid was still unknown. The objective of this study is to discuss the deodorizing activity of chewing betel quid, by using gas chromatography for methyl mercaptan odor.

MATERIALS AND METHODS

Materials. Fresh areca fruit and *Piper betle* inflorescence were purchased from a farm in Nantou County, Taiwan. They were either used immediately or stored at 4 °C until use. Red lime paste (a mixture of catechu (extract prepared from the heartwood of Acacia catechu), various herb extracts, and slaked lime) was obtained from Taichung City, Taiwan. Betel quid was composed of fresh green areca fruit, *Piper betle* inflorescence, and red lime paste (80.5:12.5:7, weight ratio).

Preparation of the Mixture of Ingredients. By our previous preparation (*23*), either two or three ingredients of betel quid were combined. The weight ratios were 80.5:12.5:7 for areca fruit, *Piper betle* inflorescence, and red lime paste; 6.44:1 for areca fruit and *Piper betle* inflorescence; 11.5:1 for areca fruit and red lime paste; and 1.79:1 for *Piper betle* inflorescence and red lime paste.

Preparation of the Testing Extracts. Portions (20 g) of various mixtures of the ingredients were extracted with 80 mL of 0.1 M phosphate buffer (pH 7.5) in a Waring blender for 3 min, followed by incubation in a 37 °C water bath for 5 min. After filtration, the volume of filtrate was adjusted to 100 mL with the same phosphate buffer used to obtain the testing extracts.

Condensed Tannin and Noncondensed Tannin Fractionation. The condensed tannin and noncondensed tannin

* To whom correspondence should be addressed. Tel: 886-4-23248174. Fax: 886-4-24755984. E-mail: wck@mercury.csmc.edu.tw.

10.1021/jf000433l CCC: $20.00
Published on Web 03/09/2001

fractions were separated from crude phenolic extract of areca fruit by our previous work (*21*).

Extraction of Crude Phenolics from Areca Fruit. A crude phenolic extract of whole areca fruit was prepared according to the method described in our previous work (*24*). In brief, areca fruit were extracted with 80% aqueous acetone (1:10, w/v) in a Waring blender for 3 min, followed by soaking for 20 min before filtration. The filtrate, containing the phenolic extract, was evaporated to dryness under reduced pressure, and the dry material was crude phenolic extract.

Alkaline Treatment of Phenolics. The crude phenolic extract was dissolved in 32 mL of propan-2-ol/water (1:1), and the pH was adjusted to 8, 10, and 12, respectively. The system was refluxed at 100 °C for 30 min. During this time, the original amber colored changed to scarlet red. After the reaction mixture was cooled, it was extracted with 120 mL of ethyl acetate to get the organic phase. The ethyl acetate extracting residue was washed with 10-mL portions of n-amyl alcohol/benzene (1:1), benzene, and ether, and the aqueous layer was evaporated to dryness under reduced pressure to obtain the aqueous phase.

Gel Filtration on Sephadex G-25. Sephadex G-25 gel was used to separate the original phenolics or alkaline-treatment phenolics by molecular size according to the method of Somers (*28*).

Determination of Total Phenolics and Condensed Tannin. The contents of total phenolics and condensed tannin were determined by the method of Julkunen-Tiitto (*29*).

Effect on the Volatility of Methyl Mercaptan. The deodorizing activity of each testing extract against methyl mercaptan was assayed by the headspace test according principally to Kita et al. (*12*). A 1-mL aliquot of crude phenolics or testing extract (ethanol or phosphate was used as control) and 1 mL of ethanolic methyl mercaptan solution (4 μg/mL) were put into a 13-mL tube (10 mm i.d. × 70 mm height). The test tube was sealed with a silicone cap, stirred for 5 s with a vortex mixer, and then incubated at 37 °C for 6 min. The volume of injected headspace was 0.5 mL, and the manual injection was carried out with a gastight syringe. The content of methyl mercaptan in the headspace under the above incubation was determined by gas chromatography with a flame photometric detector (Perkin-Elmer n-930-0061). Following are the gas chromatography conditions used: instrument, Perkin-Elmer GC autosystem; column temperature, 50 °C; column, 1.8 m × 3.2 mm, Ni-lined tube packed with 5% polyphenyl ether 5 ring on Chromosorb WAW DMCS 80−100 mesh; carrier gas (nitrogen) velocity, 20 mL/min; injector temperature, 90 °C; air velocity, 149 mL/min; hydrogen velocity, 77 mL/min. The deodorizing activity (DA) was calculated from the following equation: $DA = (C - S)/C \times 100\%$, where C is CH_3SH peak area of control and S is CH_3SH peak area after incubation with a sample. The calibration step was carried out by using the above model to estimate the effect of different amounts of dry matter of betel quid extract, and the linearity is about 95%.

Preparation and Storage of Methyl Mercaptan Stock Solution. The stock solution (20 μg/mL) was prepared by mixing 2 mL of original methyl mercaptan (1 mg/mL benzene) and 98 mL of alcohol (99.5%), and was stored at −20 °C. The experimental concentration was prepared by diluting the stock solution with alcohol (99.5%).

Removal of Phenolics by Polyvinylpolypyrrolidone (PVPP). Phosphate buffer extract of betel quid (80 mL) was mixed with various amounts of PVPP (0.1, 0.5, 1, 2, or 3 g) under room temperature. After 1 h, the mixture was centrifuged to remove the residues, and the final volume was adjusted to 80 mL with phosphate buffer again.

RESULTS AND DISCUSSION

To evaluate the deodorizing activity, measurement of the depression on the volatility of methyl mercaptan was performed. Red lime paste is one kind of alkaline ingredient, and it is mainly composed of calcium hydroxide and other herb extracts. To avoid the alkaline interruption on the volatility of methyl mercaptan, different controls (the same pH for each sample) were made to reveal the actual effect. As illustrated in Table 1, the single red lime paste and the mixture of *Piper betle* inflorescence and red lime paste showed the best deodorizing activities (100%), and were followed by the mixture of areca fruit and red lime paste (90%), whole betel quid (86%, areca fruit, *Piper betle* inflorescence, and red lime paste), mixture of areca fruit and *Piper betle* inflorescence (68%), areca fruit alone (40%), and *Piper betle* inflorescence alone (12%).

Table 1. Deodorizing Activity of Phosphate Buffer Extracts from the Ingredients of Betel Quid

sample[a]	deodorizing activity[b] (%)
AF	40 ± 3^{d}
PI	12 ± 4^{e}
RL	100 ± 2^{a}
calcium hydroxide	2 ± 1^{a}
AF+PI	68 ± 7^{c}
AF+RL	90 ± 2^{b}
PI+RL	100 ± 1^{a}
AF+PI+RL	86 ± 4^{b}

[a] AF, areca fruit; PI, *Piper betle* inflorescence; RL, red lime paste; AF + PI, mixture of AF and PI at the weight ratio of 6.44:1; AF + RL, mixture of AF and RL at the weight ratio of 11.5:1; PI + RL, mixture of PI and RL at the weight ratio of 1.79:1; AF + PI + RL, mixture of AF, PI, and RL at the weight ratio of 80.5:12.5:7. [b] Data bearing different superscript letters were significantly different ($p < 0.05$).

In Taiwan, red lime paste is the most popular lime paste used in betel quid. However, the ingredients of red lime paste are very complex. Slaked lime (calcium hydroxide), catechu, licorice concentrate, some herb extracts, and additives are the usual ingredients of red lime paste. Calcium hydroxide is the major and the most important substance of red lime paste, so we also evaluated the effect of calcium hydroxide alone. Only very weak effect on the volatility of methyl mercaptan was found. From this result, we found that the deodorizing activity probably came from the other complex ingredients (catechu or herb extracts). Red lime paste, or the mixture of *Piper betle* inflorescence and red lime paste, was never chewed alone. It clearly indicated that betel quid showed perfect inhibitory effect on the volatility of methyl mercaptan.

Areca fruit is the main ingredient of betel quid, and it exhibited lower depression on the volatility of methyl mercaptan. However, the mixture of areca fruit and red lime paste showed strong deodorizing activity. To separately understand the function of pure calcium hydroxide and red lime paste, they were added into areca fruit respectively to further identify the role of red lime paste on the deodorizing activity of areca fruit. To easily compare and lower the effect of various ratios of red lime paste (or calcium hydroxide), the concentration of the mixing extract of the areca fruit and red lime paste (or calcium hydroxide) (in Figure 1) is prepared as only one-fifth of that in Table 1. As shown in Figure 1, the deodorizing activity was increased with the higher pH and elevated ratios of red lime paste (or calcium hydroxide) to areca fruit. It showed that high levels of alkaline material added into areca fruit significantly promoted the deodorizing activity. All results about the deodorizing activity expressed for samples that included red lime paste or calcium hydroxide in the study were obtained by comparing with the results of related control samples (red lime paste or calcium hydroxide). Methyl

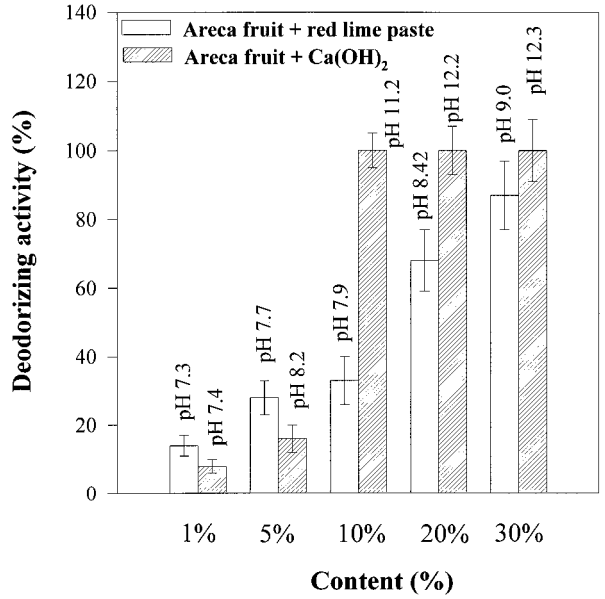

Figure 1. Effect of calcium hydroxide or red lime paste added into areca fruit on the deodorizing activity.

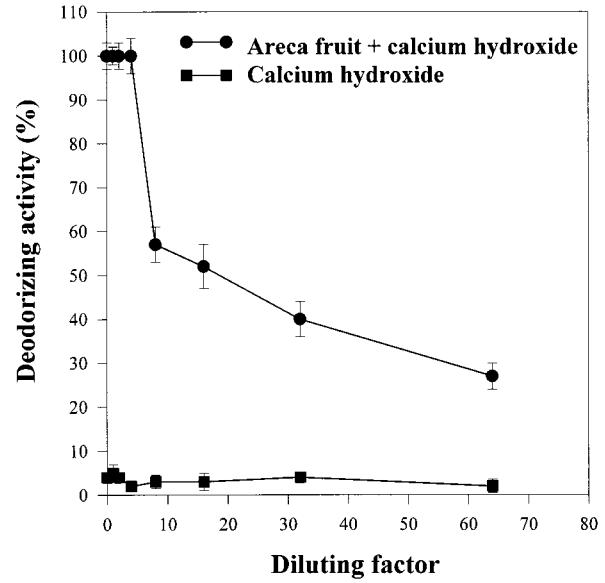

Figure 2. Deodorizing activity of serial dilutions obtained from the extract of the mixture of areca fruit and calcium hydroxide (20 wt % of areca fruit) or calcium hydroxide alone with pH 12.2 of calcium hydroxide solution.

mercaptan is unstable at basic pH levels. Disulfide bonds are easily formed. To further reveal the actual effect, the inhibitory effects for the mixing extract (pH 12.2) of areca fruit and 20% calcium hydroxide were performed by diluting with the calcium hydroxide solution (pH 12.2). To identify the role of calcium hydroxide on the volatility of methyl mercaptan, the same amount of calcium hydroxide (4 g in 100 mL) solution was diluted with calcium hydroxide solution (pH 12.2). As shown in Figure 2, calcium hydroxide showed no inhibitory effect on the volatility of methyl mercaptan. The deodorizing activities decreased with the increasing dilutions, and the decrease in the deodorizing activity is not owing to the drop in calcium hydroxide. It is clear that the mixing extract of areca fruit and calcium hydroxide contained components which exhibited actual inhibitory effect on the volatility of methyl mercaptan.

Table 2. Deodorizing Activity of Phosphate Buffer Extract of Betel Quid after PVPP Treatment

amount of PVPP (g)	deodorizing activity[a]
nonaddition	96 ± 0.3^{a}
0.1	69 ± 1.0^{b}
0.5	32 ± 2.4^{c}
1.0	27 ± 3.6^{d}
2.0	17 ± 2.3^{e}
3.0	7 ± 3.5^{f}

[a] Data bearing different superscript letters were significantly different ($p < 0.05$).

Table 3. Contents of Total Phenolics and Condensed Tannin in the Phosphate Buffer Extract of Betel Quid after PVPP Treatment

	content (mg/g of fresh wt)	
amount of PVPP (g)	total phenolics[a,c]	condensed tannin[b,c]
nonaddition	10.10 ± 0.55^{ac}	3.65 ± 0.23^{a}
0.1	6.85 ± 0.31^{b}	2.30 ± 0.03^{b}
0.5	4.70 ± 0.21^{c}	1.55 ± 0.04^{c}
1.0	3.20 ± 0.15^{d}	1.00 ± 0.03^{d}
2.0	2.10 ± 0.10^{e}	0.75 ± 0.05^{e}
3.0	1.25 ± 0.12^{f}	0.50 ± 0.02^{f}

[a] mg gallic acid equivalent per gram of sample weight. [b] mg catechin equivalent per gram of sample weight. [c] Data bearing different superscript letters within the same column were significantly different ($p < 0.05$).

Many natural phenolics are found to have deodorizing activity (*14*−*16*). The role of phenolics on the deodorizing activity of betel quid was determined by the treatment of PVPP in this study. The deodorizing activity was significantly decreased by the addition of higher levels of PVPP (Table 2). The contents of total phenolics and condensed tannin were also found to decrease with the increasing amounts of PVPP treatment (Table 3). It is clear that phenolics probably play an important role on the deodorizing activity. Areca fruit, the major ingredient of Taiwanese betel quid, contained abundant phenolics (*24*). However, the crude phenolic extract from areca fruit and their condensed tannin and noncondensed tannin fractions showed only weak inhibition on the volatility of methyl mercaptan. To further understand the effect of alkaline treatment on the deodorizing activity of the phenolics, the crude phenolic extract of areca fruit was refluxed under alkaline conditions (NaOH, pH 13) at 100 °C for 30 min. Both organic and aqueous phases were separated from the refluxing product by the extracting procedures described previously. As illustrated in Figure 3, the deodorizing activity appeared mainly in the aqueous phase and showed a dose-dependent manner. The deodorizing activity was also greatly influenced by the alkalinity and time of refluxing. Results in Figure 4 show that the higher the pH of refluxing reaction, the better the deodorizing activity. The deodorizing activity was also found well-correlated with the refluxing time (Figure 5). These results indicated clearly that original phenolics of areca fruit were changed to something with excellent deodorizing activity and phenolic characteristics after highly alkaline treatment.

To identify the effectively deodorizing components in the aqueous phase, Sephadex G-25 gel packing column was used. As shown in Figure 6, the original phenolic extract was fractionated into four moieties and two moieties for the aqueous phase of the refluxing product. Our previous study (*30*) showed that the molecular size in the two moieties of the aqueous phase of the refluxing product was greater than that of those in the original

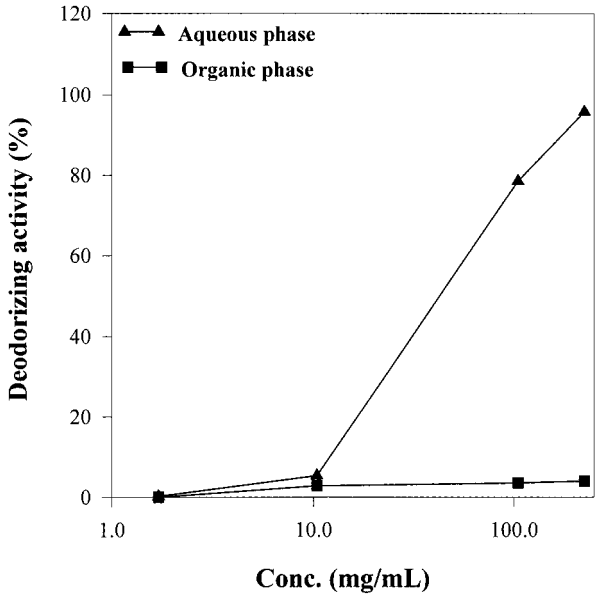

Figure 3. Deodorizing activity of the aqueous and organic phases of the crude phenolic extract from areca fruit after alkaline treatment (NaOH, pH 13; at 100 °C for 30 min).

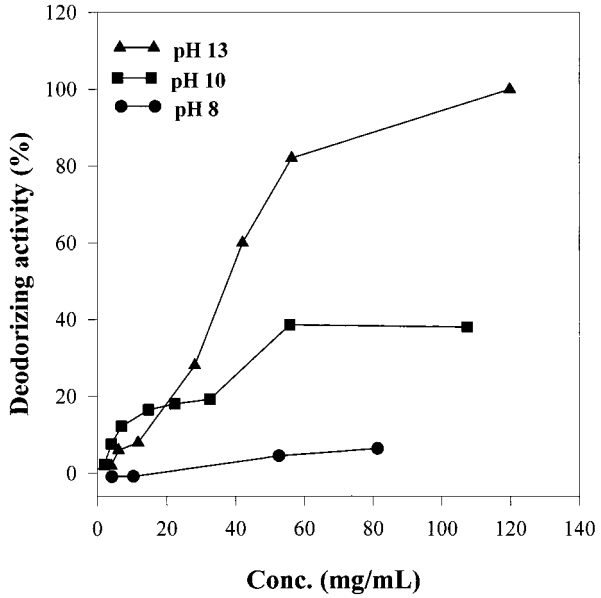

Figure 4. Deodorizing activity of the aqueous phase of the crude phenolic extract from areca fruit after various alkaline treatments (NaOH, pH 8, 10, 13; at 100 °C for 30 min).

phenolic extract. In addition, the fractions are also found in the mixture of areca fruit and red lime paste under 37 °C water bath. Evidently, the higher molecular size of particles should be the major contributor of deodorizing activity of betel quid.

Betel quid effectively inhibited the volatility of methyl mercaptan, and slaked lime paste played an important role in this function. Our previous study found that the crude phenolics of areca fruit showed perfect antioxidant and antimutagenic activities. However, the deodorizing activity was found only in the aqueous phase of areca fruit after alkaline treatment. Further studies about the characteristics and biological activities of the aqueous phase of areca fruit after alkaline treatment will be required. Also, a clinical examination for the halitosis removal will be necessary.

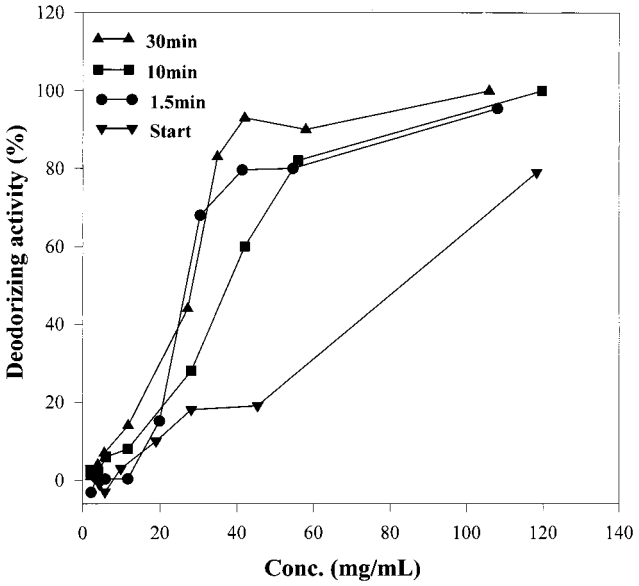

Figure 5. Deodorizing activity of the aqueous phase of the crude phenolic extract from areca fruit after alkaline treatment of various refluxing times (NaOH, pH 13; at 100 °C for 0, 1.5, 10, and 30 min).

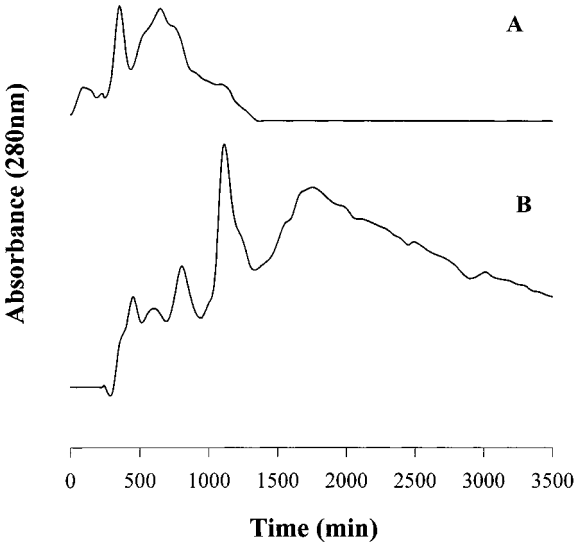

Figure 6. Separation by gel filtration (Sephadex G-25) of the aqueous phase of the crude phenolic extract from areca fruit after alkaline treatment (NaOH, pH 13; 100 °C for 30 min) (A), and crude phenolic extract of areca fruit (B).

LITERATURE CITED

(1) Chen, H. C.; Chang, Y. S.; Lee, T. C. The mutagenicity of nitrite-treated aqueous extract of *Piper betle* L. *Proc. Natl. Sci. Counc., Repub. China, Part B: Life Sci.* **1984**, *8*, 4−10.

(2) Kwan, H.-W. A statistical study on oral carcinomas in Taiwan with emphasis on the relationship with betel nut chewing: a preliminary report. *J. Formosan Med. Assoc.* **1976**, *75*, 497−505.

(3) IARC. Betel-quid and areca-nut chewing. In *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*; Vol. 37; International Agency for Research on Cancer: Lyon, France, 1985; pp 141−202.

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

(5) Hwang, L. S.; Wang, C.-K.; Sheu, M.-J.; Kao, L.-S. Phenolic compounds of *Piper betle* flower as flavoring and neuronal activity 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.

(6) Hwang, L. S.; Wang, C.-K.; Kao, L.-S. Neuronal activity modulating components in betel quid. Presented at Symposium on Betel Quid Chewing and Its Health Effect, Kaohsiung, Taiwan, 1993.

(7) Tonzetich, J.; Richter, V. J. Evaluation of volatile odoriferous components of saliva. *Arch. Oral Biol.* **1964**, *9*, 39−45.

(8) Tsunoda, M. Analysis of fetor exo by gas chromatography. *Nippon Shishubyo Gakkai Kaishi* **1975**, *17*, 1−13.

(9) Tokita, F.; Ishikawa, M.; Shibuya, K.; Koshimizu, M.; Abe, R. Deodorizing activity of some plant extracts against methyl mercaptan. *Nippon Nogeikagaku Kaishi* **1984**, *58*, 585−589.

(10) Miura, K.; Inagaki, T.; Nakatani, N. Sturcture and activity of new deodorant biphenyl compounds from thyme (*Thymus vulgaris* L.). *Chem. Pharm. Bull.* **1989**, *37*, 1816−1819.

(11) Nakatani, N.; Miura, K.; Inagaki, T. Structure of new deodorant biphenyl compounds from thyme (Thymus vulgaris L.) and their activity against methyl mercaptan. *Agric. Biol. Chem.* **1989**, *53*, 1375−1381.

(12) Kita, N.; Fujimoto, K.; Nakajima, I.; Hayashi, R.; Shibuya, K. Screening test for deodorizing substances from marine algae and identification of phlorotannins as the effective ingredients in *Eisenia*. *J. Appl. Phys.* **1990**, *2*, 155−162.

(13) Tsunoda, M.; Sato, H.; Ohkushi, T. The experimental study for the effect of sodium copper chlorophyll in halitosis. *Nippon Shishubyo Gakkai Kaishi* **1981**, *23*, 490−498.

(14) Kaji, A. Production methods of air-cleaners. *Japan Patent* 51-27882, 1976.

(15) Sato, Y.; Terazawa, M.; Ishikawa, H. Deodorant gums containing green tea flavonoids. *Shokuhinkogyo* **1984**, *27*, 42−48.

(16) Suzuki, S.; Moroe, M.; Uchida, Y. Green tea flavonoids. *Shokuhinkogyo* **1983**, *26*, 57−65.

(17) Grabowski, J.; Brown, A.; Bruza, D.; Sitaram, N. Heart rate change following sleep infusion of arecoline. *Psychiatry Res.* **1987**, *22*, 261−262.

(18) Soncrant, T. T.; Holloway, H. W.; Greig, N. H.; Rapoport, S. I. Reginol brain metabolic responsivity to the muscarinic cholinergic agonist arecoline is similar in young and aged Fischer-344 rats. *Brain Res.* **1989**, *487*, 255−258.

(19) Soncrant, T. T.; Raffaele, K. C.; Asthana, S.; Berardi, A.; Morris, P. P.; Haxby, J. V. Memory improvement without toxicity during chronic, low dose intravenous arecoline in Alzheimer's disease. *Psychopharmacology* **1993**, *112*, 421−427.

(20) Maiese, K.; Holloway, H. H.; Larson, D. M.; Soncrant, T. T. Effect of acute and chronic arecoline treatment on cerebral metabolism and blood flow in the conscious rat. *Brain Res.* **1994**, *641*, 65−75.

(21) 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.

(22) Wang, C.-K.; Wu, M.-J. The separation of phenolics from *Piper betle* leaf and the effect on the mutagenicity of arecoline. *J. Chin. Agric. Chem. Soc.* **1996**, *34*, 638−647.

(23) Wang, C.-K.; Hwang, L. S. The effect of betel quid on catecholamine secretion from adrenal chromaffin cells. *Proc. Natl. Sci. Counc., Rep. China, Part B: Life Sci.* **1997**, *21*, 129−136.

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

(25) Wang, C.-K.; Hwang, L. S. Phenolic compounds of betel quid chewing juice. *Food Sci.* **1993**, *20*, 514−526.

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

(27) Wang, C.-K.; Peng, C.-H. The mutagenicities of alkaloids and N-nitrosoguvacoline from betel quid. *Mutat. Res.* **1996**, *360*, 165−171.

(28) Somers, T. C. Wine-tannins isolation of condensed flavonoid pigments by gel-filtration. *Nature* **1966**, *209*, 368−370.

(29) 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.

(30) Wang, C.-K.; Yen, H.-C. The biological and characteristic properties of areca fruit polyphenols. In *Polyphenols, Wine and Health Communications*; Cheze, C., Vercauteren, J., Eds.; Polyphenols, Wine and Health Symposium: Bordeaux, France, 1999; pp 3−4.

Received for review April 5, 2000. Revised manuscript received January 23, 2001. Accepted January 24, 2001. This research work was partly supported by the National Science Council, Republic of China (under Grant NSC 86-2314-B040-002) and partly supported by Chung Shan Medical & Dental College (under Grant CSMC 82-B005).

JF000433L