Antioxidant Properties of Phenolic Compounds in Macadamia Nuts

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ABSTRACT: Phenolic compounds in macadamia nuts and shells were identified, and their antioxidant activities were evaluated in refined macadamia nut oil. Thin-layer chromatography of oil extracted from macadamia nut kernels and shells indicated the possible presence of catechol, phrogallol, and 3,4,5-trihydroxy phenolic compounds. Four phenolic compounds were tentatively identified as 2,6-dihydroxybenzoic acid, 2′-hydroxy-4′-methoxyacetophenone, 3′,5′-dimethoxy-4′-hydroxyacetophenone, and 3,5-dimethoxy-4-hydroxycinnamic acid. Adding 0.01% of 2′-hydroxy-4′-methoxyacetophenone, 3′,5′-dimethoxy-4′-hydroxyacetophenone, or 3,5-dimethoxy-4-hydroxycinnamic acid to the oil significantly increased the Rancimat induction time against the control ($P < 0.0001$). At this concentration, 3′,5′-dimethoxy-4′-hydroxyacetophenone was the most effective compound added. Although induction times of oils with 0.01% 2,6-dihydroxybenzoic acid were not significantly different from the control, at 0.1% there was a significant difference from the control ($P < 0.0001$). The activity was the same as that of 2′-hydroxy-4′-methoxyacetophenone at 0.1%. *JAOCS 73,* 1585–1588 (1996).

KEY WORDS: Antioxidant, macadamia nuts, oxidation, phenolic compounds.

The factors that determine the oxidative stability of macadamia nuts are still largely unknown. In studies on storage of macadamia nuts, Tsumura (1) reported that the tocopherol level (6.4–18 μg/g dry matter) was so low that it did not contribute to kernel stability. Differences in the fatty acid composition were not great enough to contribute to differences in stability (2). High moisture conditions accelerated deterioration of nut quality (3).

Because addition of antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), increased storage life of roasted macadamia nuts (4), perhaps natural antioxidants present in the nuts contribute to the oxidative stability of macadamia nuts. Phenolic compounds can act as coloring, antioxidant, and anticarcinogenic compounds in foods (5,6). In olive oil, phenolic compounds act as antioxidants and extend the oil shelf-life (7). Although phenolics have been identified and quantified in many foods, macadamia nuts have neither been tested for phenolics nor have the antioxidative properties of these compounds been tested in macadamia nut oils.

The objectives of this study were to identify and quantitate phenolic compounds in macadamia nuts and shells and to compare the antioxidant activity of the identified phenolic compounds at various levels in refined macadamia nut oil.

EXPERIMENTAL PROCEDURES

Oil extraction. Macadamia nuts, Hawaii Agricultural Experiment Station variety HAES 246 (Keauhou) and Purvis varieties from Waimanalo Experiment Station in Hawaii, were harvested during November and December 1993 and dried. Nuts were cracked on a macadamia nut cracker, made by the University of Hawaii Agriculture Engineering Dept., and oil was extracted just prior to analysis. The kernels were chopped with a Cuisinart Food Processor (Cuisinart, Inc., Greenwich, CT) for 10–15 s. The shells were ground into 2–3 mm diameter particles with a Waring commercial blender (Waring Products Co., McConnellsburg, PA). Approximately 80 g of chopped kernels or chopped kernels plus shells were put into a 20 × 20 cm organdy cloth, which had been previously cleaned with ethyl acetate [high-performance liquid chromatography (HPLC) grade; Fisher Scientific, Pittsburgh, PA]. The nuts and cloth were pressed at 15,000 psi in the cell of a Carver Laboratory Press (Fred S. Carver, Inc., Summit, NJ). The oil was centrifuged at 2,000 rpm for 15 min at 10–20°C on a Beckman Model J2-21 Centrifuge (Beckman Instruments, Inc., Fullerton, CA). The top clear oil was decanted into clean bottles, which were then filled with nitrogen gas, capped, and stored in the dark at 4°C until further analysis.

Phenolic extraction. Five mg of *p*-hydroxycinnamic acid (Sigma Chemical Co., St. Louis, MO) was added to 50 g of ground shells as an internal standard. The method of Tyman *et al.* (8) was used to extract the lipids from macadamia nut shells. The method of Tang and Young (9) was used to extract phenolic compounds remaining in the shell and to hydrolyze them for extraction with ethyl acetate.

To extract phenolic compounds from laboratory-pressed macadamia nut kernel oil, 20 g of oil was mixed with 5 mg of *p*-hydroxycinnamic acid as internal standard, then 20 mL of ethyl acetate (HPLC grade, Fisher Scientific) and the mixture

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was washed with 25 mL of a 5% sodium bicarbonate aqueous solution. The mixture was centrifuged in plastic bottles at 2,000 rpm at 24°C for 15 min. The oil layer was removed and saved, 20 mL of a 2% sodium hydroxide aqueous solution was added, and the mixture was shaken for 30 min. Three liquid-liquid extractions with sodium hydroxide were performed as above. The aqueous layer was filtered through grade 1 filter paper. The extracts were hydrolyzed with the method of Tang and Young (9). Both layers of the liquid-liquid extraction were tested for presence of phenolic compounds with Folia-Ciocalten reagent (Sigma Chemical Co.) by the thin-layer chomatography (TLC) method of Tang and Young (9). No phenolic compounds were detected in the extract to be discarded. The extraction process was performed in the dark to prevent phenolic compound decomposition.

Gas chromatography. The extracts were methylated with ethereal diazomethane by following the method of Tang and Young (9). One-microliter samples were injected into a 30-m nonpolar silica capillary column (DB-5; J&W Scientific, Folsum, CA) on a Hewlett Packard model 5890 Series II gas chromatograph (Hewlett Packard, Minneapolis, MN). The temperature programming, injector and detector temperatures used were the same as by Williams (10). The retention times were compared with 44 external standards for tentative identification.

TLC. The extracts were spotted on 60 F254 silica gel plates with disposable 25-μL pipettes. The mobile phase was chloroform/ethyl acetate, 3:1. The plates were developed to approximately 12 cm in 20 min, and spots were detected with short-range ultraviolet (254 and 265 nm) and sprayed with Folia-Ciocalten reagent, followed by ammonia vapor or 2% ferric chloride in alcohol (9). Phenolic compounds react with Folia-Ciocalten to produce blue colors and with ferric chloride to produce yellow, orange, green or blue colors, depending on the class of phenolic compound. Results were again compared with 44 external standards for tentative identification.

Stability tests. Oils tested included commercially prepared crude macadamia nut oil (Oils of Aloha, Waialua, HI), commercially refined macadamia nut oil (Oils of Aloha), and crude oils from Keauhou and Purvis varieties that were pressed in the laboratory with and without shells. Cavaletto *et al.* (2) reported no significant difference in the fatty acid composition of various cultivars (C16:0, 7.4; C16:1, 18.5; C18:0, 2.8; C18:1, 6.5; C18:2, 1.5; C20:4, 1.9; C20:0, 2.3). The commercial phenolic compounds, 2,6-dihydroxybenzoic acid, 2′-hydroxy-4′-methoxyacetophenone, 3′,5′-dimethoxy-4′-hydroxyacetophenone and 3,5-dimethoxy-4-hydroxycinnamic acid, (Sigma Chemical Co.) were added individually to commercially refined macadamia nut oil at concentrations of 0.01, 0.1, 0.25, or 1%. The phenols were checked for purity by gas chromatography, mixed with 2–5 mL of ethyl acetate, then added to the oils. Ethyl acetate was evaporated under nitrogen. Oil stability was measured on a Rancimat 617 Series 03 (Metrohm, Inc., Herisau, Switzerland). Oil (2.5 g) was weighed directly into the reaction vessels and heated to 130°C. Air flow rate was 20 L/h. The increase in conductivity was measured against time, and the point at which the slope changed was determined as the end of the induction period. Three replications of each sample were averaged, and results were analyzed by the *t*-test and analysis of variance. Least significant differences were calculated.

RESULTS AND DISCUSSION

Table 1 shows the compounds identified in both kernel oil and shell. Although the chromatogram contained over 30 peaks, the only 4 that matched with the 44 external standards were 2,6-dihydroxybenzoic acid; 2′-hydroxy-4′-methoxyacetophenone; 3′,5′-dimethoxy-4′-hydroxyacetophenone and 3,5-dimethoxy-4-hydroxycinnamic acid, which are fairly common phenolic compounds (5,6). Further research on the other unidentified compounds may reveal more unusual phenolic compounds. The retention times for peaks on kernel oil and shell chromatograms were similar. The total concentration of the four tentatively identified phenolic compounds in the shells was 17 times higher than in the kernels (Table 1). The concentration of the individual phenolic compounds in the shells varied from 12 to 36 times higher than in the kernels (Table 1).

The four phenolic compounds were also identified by thin-layer chromatography. In addition, 14 other spots in both oil and shell extracts reacted with ferric chloride to produce blue, blue-black, green, yellow, and brown colors. According to Van Sumere (11), blue represents phrogallol derivatives, blue-black represents 3,4,5-trihydroxy phenolic compounds, and green represents catechol. Although the method cannot be used for positive identification, it may indicate the presence

TABLE 1
Concentration of Phenolic Compounds Tentatively Identified in Macadamia Nut Kernel Oil and Shell by Gas Chromatography

Phenols	Concentration in kernel oil (μg/g)	Concentration in shell (μg/g)
2,6-Dihydroxybenzoic acid	24.0 ± 3.61[a]	285.6 ± 56.9
2′-Hydroxy-4′-methoxyacetophenone	6.9 ± 0.9	83.7 ± 27.1
3′,5′-Dimethoxy-4′-hydroxyacetophenone	10.5 ± 0.7	202.3 ± 47.0
3,5-Dimethoxy-4-hydroxycinnamic acid	7.3 ± 0.3	266.3 ± 22.6
Total concentration	48.7	837.9

[a]Average concentration plus or minus SD.

TABLE 2
Effect of Phenolic Compounds on Rancimat Induction Time (h) at 130°C in Commercially Refined Macadamia Nut Oil

Concentration (%)	Induction time (h)			
	A[a]	B	C	D
0	0.50 ± 0.03a[b,c]	0.50 ± 0.03a	0.50 ± 0.03a	0.50 ± 0.03a
0.01	0.59 ± 0.06aε[d]	1.01 ± 0.09bδ	1.34 ± 0.4bϕ	1.08 ± 0.02bδ
0.10	1.10 ± 0.11bγ	1.14 ± 0.01cγ	N/A	N/A
0.25	N/A[e]	N/A	3.58 ± 0.1c	N/A
1.00	10.67 ± 0.06c	N/A	N/A	N/A

[a]A = 2,6-dihydroxybenzoic acid; B = 2′-hydroxy-4′-methoxyacetophenone; C = 3′,5′-dimethoxy-4′-hydroxyacetophenone; D = 3,5-dimethoxy-4-hydroxycinnamic acid.
[b]Average Rancimat induction time at 130°C in hours ± SD.
[c]Means in the same column with different letters are significantly different from each other ($P < 0.0001$).
[d]Means in the same row with different Greek letters are significantly different from each other ($P < 0.0001$).
[e]Not available.

of other catechols, phrogallol, and 3,4,5-trihydroxy phenolic compounds.

Table 2 shows the average Rancimat induction time of refined macadamia nut oil with and without added phenolic compounds, identified in macadamia nut kernels and shells. Some of the phenolic compounds were difficult to dissolve in the oil, so testing higher concentrations was not possible for all identified phenolic compounds. The control had a short induction time of 0.5 h. Compound A, 2,6-dihydroxybenzoic acid, did not significantly increase the induction time compared to the control at 0.01%, but did significantly increase ($P < 0.0001$) the induction time at 0.10% and had the same antioxidant activity as 2′-hydroxy-4′-methoxyacetophenone. Adding 0.01% of 2′-hydroxy-4′-methoxyacetophenone, 3′,5′-dimethoxy-4′-hydroxyacetophenone, or 3,5-dimethoxy-4-hydroxycinnamic acid to the oil significantly increased ($P < 0.0001$) the Rancimat induction time compared to the control. At 0.01% concentration, 3′,5′-dimethoxy-4′-hydroxyacetophenone was significantly more effective ($P < 0.0001$) than 2′-hydroxy-4′-methoxyacetophenone, 3,5-dimethoxy-4-hydroxycinnamic acid, or 2,6-dihydroxybenzoic acid.

In macadamia nut oil, natural concentrations of 2,6-dihydroxybenzoic acid; 2′-hydroxy-4′-methoxyacetophenone; 3′,5′-dimethoxy-4′-hydroxyacetophenone, and 3,5-dimethoxy-4-hydroxycinnamic acid were 0.0024, 0.0007, 0.0001, and 0.0007%, respectively, with a total concentration of 0.004%. Although this is half the lowest concentration tested for antioxidant activity in this study, the synergistic effects or the effect of other unidentified phenolic compounds is not known.

In addition, processing may have an effect on the phenolic compounds present in the oil. In our study, kernels and shells were extracted separately and tested for phenolic compounds; however, in the industry, macadamia nut oil is cold-pressed with shell fragments. The amount of phenolic compounds that may dissolve in the oil during pressing is unknown. Rancimat induction times were significantly longer ($P < 0.0001$) in both oils that were pressed with the shell than in oils that were pressed without shell (Table 3). The induction time of commercial crude oil was significantly greater ($P < 0.0001$) than the induction time of refined oil (Table 3). Refining may remove the slightly acidic phenolic compounds when free fatty acids are removed.

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TABLE 3
Rancimat Induction Time at 130°C of Macadamia Nut Oils

	Commercial oil	Refined oil	Keauhou		Keauhou and Purvis	
			With shell	Without shell	With shell	Without shell
Induction time (h)	5.48 ± 0.27[a]a[b]	0.50 ± 0.03b	9.21 ± 0.06c	8.70 ± 0.26d	3.05 ± 0.07e	2.13 ± 0.05f

[a]Average induction time ± SD.
[b]Values in the same row with different letters are significantly different from each other ($P < 0.0001$).

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