Effects of Flavonoids on Thermal Autoxidation of Palm Oil: Structure-Activity Relationships

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The effects of several flavonoids and other known antioxidants on the thermal autoxidation of refined, bleached and deodorized (RBD)-palm oil were studied. The lipid peroxidation was indexed by measuring the malonyldialdehyde (MDA) production using the 2-thiobarbituric acid (TBA) test. The antioxidative action decreased in the order of morin > kaempferol > myricetin > quercetin > vitamin A > α-tocopherol > apigenin > (+)-catechin > chrysin > datiscetin > luteolin > naringin > taxifolin > rutin > butylated hydroxytoluene (BHT) > naringenin.

The flavonoid aglycones were more potent in their antiperoxidative action than their corresponding glycosides. Structure-activity revealed that the flavonoid molecule with polyhydroxylated substitutions on rings A and B, a 2-3 double bond, a free 3-hydroxyl substitution and a 4-keto moiety, would confer potent antiperoxidative properties upon the compound. The flavonols, namely morin, myricetin, kaempferol and quercetin, would be suitable potential antioxidants for use in the stabilization of RBD-palm oil and its fractions against thermal autoxidation. The structural activity of the flavonoids on the RBD-palm oil was similar to those observed for these compounds in animal tissue or enzyme systems.

Palm oil and its fractions, palm olein and palm stearin, are gaining importance in the food industry (1). Palm stearin is used instead of beef-tallow for the manufacturing of margarines (2,3). Palm oil liquid fractions are used as frying media in household and industrial friers and have been compared to standard edible oils and fats, such as soybean, groundnut, sunflower, rapeseed and tallow (4). During the life of a frying oil the development of brown color is normally associated with oxidation and polymerization.

Deterioration of lipid compounds is expedited by increase in acidity, oxidation and contamination of trace metals. The thermal autoxidation of crude palm oil in the presence of various metal catalysts has been studied (5). However, the study of thermal autoxidation of refined, bleached and deodorized (RBD)-palm oil has not been reported.

Flavonoid substances, which occur naturally in plants, are recognized as important compounds in conferring stability towards autoxidatin on the lipids of vegetable source (6–9). The present project was carried out in order to select an appropriate antioxidant from the flavonoid group of compounds for their possible use in the stabilization of RBD-palm oil. Their activity in relation to their structures was also investigated.

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METHODS AND MATERIALS

RBD-palm oil was from Lam Soon Oil and Soap Manufacturing (S) Pte., Ltd., Singapore. 2-Thiobarbituric acid (TBA) was purchased from Sigma Chemical Company (St. Louis, MO). The flavonoids used in this study (Table 1) were obtained from Extrasynthese, Lyon-Nord, Genay, France. Other chemicals used were obtained from E. Merck, Darmstadt, Germany.

Purified oxygen was supplied by Singapore Oxygen Air Liquide, Pte., Ltd. They assayed the oxygen cylinder using Singapore Standard No. 153 (similar to USA recommendations) and found it to contain oxygen, 99.8%; water, less than 3 ppm; hydrogen, 1 ppm; CO_2, less than 1 ppm; hydrocarbon, 25 ppm; N_2 + Argon, less than 0.2%.

The standard solutions and buffer were prepared in an oxygen-free nitrogen atmosphere. The water used for Tris-KCl buffer was ultra pure distillate obtained from SYBRON BARNSTEAD, nano pure II ion-exchanger and was free from trace metals, organics and dissolved oxygen. The absolute ethanol used in the preparation of the standards was double glass distilled and then desiccated until use.

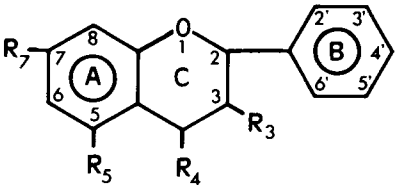
Standard antioxidant solutions. Different flavonoid (10 mM) stock solutions were prepared using absolute ethanol as solvent. The working solution was 0.03 mM for each flavonoid. Similar concentrations (0.03 mM) were used for the other antioxidants, vitamin A, α-tocopherol and butylated hydroxytoluene (BHT). The analytical data of the palm oil used before and after refining is given in Table 2.

Thermal autoxidation of palm oil: Oxidation procedure. Four ml of Tris/KCl buffer (pH = 7.4) was added to 2 ml of RBD-palm oil. This was vortex-mixed for 2 min and placed in a water-bath (80°C). Oxygen gas was bubbled into the mixture for 40 min at the rate of 20 ml/min. Simultaneous experiments were also carried out using palm oil spiked separately with 30 μl of ethanolic solutions containing (0.03 mM) quercetin, myricetin, naringin, naringenin, rutin, morin, apigenin, chrysin, luteolin, datiscetin, kaempferol, taxifolin, (+)-catechin, BHT, vitamin A (retinol) and α-tocopherol, respectively. The control tube contained only absolute ethanol (30 μl) plus RBD-palm oil and buffer. The experiments were carried out in quadruplicate. At the end of the thermal autoxidation of the palm oil, all tubes were flushed with oxygen-free nitrogen before cooling to preclude continued oxidation resulting from any oxygen gas remaining in the head space of the tubes. All the tubes were cooled and centrifuged at 2000 rpm for 10 min.

Aliquots (1 ml) of the clear aqueous layer were removed and placed into separate test-tubes and subjected to the malonyaldehyde (MDA) estimation. The amount of peroxides and related compounds formed in the thermal autoxidation of RBD-palm oil was deter-

TABLE 1

Chemical Structure of Flavonoids Studied



Compounds	Substituent positions							
	Ring A		Ring C		Ring B			
	R5	R7	R3	R4	2′	3′	4′	5′
Flavones:								
Apigenin	OH	OH	H	O	H	H	OH	H
Chrysin	OH	OH	H	O	H	H	H	H
Luteolin	OH	OH	H	O	H	OH	OH	H
Flavonols:								
Datiscetin	OH	OH	OH	O	OH	H	H	H
Quercetin	OH	OH	OH	O	H	OH	OH	H
Myricetin	OH	OH	OH	O	H	OH	OH	OH
Morin	OH	OH	OH	O	OH	H	OH	H
Kaempferol	OH	OH	OH	O	H	H	OH	H
Rutin	OH	OH	O-rutinose	O	H	OH	OH	H
Flavanones:								
Naringenin	OH	OH	H,H	O	H	H	OH	H
Naringin	OH	OH	O-rhamnogluc.	O	H	H	OH	H
Flavanonols:								
Taxifolin (Dihydroquercetin)	OH	OH	H,OH	O	H	OH	OH	H
Flavan-3-ols:								
(+)-Catechin	OH	OH	H,OH	H,H	H	OH	OH	H

mined using our earlier method (9). Briefly, 1 ml (0.75%) of thiobarbituric acid (TBA) was added to 1 ml of aqueous buffer solution containing dissolved peroxides and the reaction mixture was vortex-mixed for 2 min before developing the color in a boiling water-bath for 10 min. The final color developed was read at 534 nm using a Shimadzu 260 UV spectrophotometer. The amount of MDA formed was calculated using the molar extinction coefficient $1.56 \times 10^5 M\ cm^{-1}$.

RESULTS

The different amounts of MDA formation induced by the thermal autoxidation of palm oil are presented in Table 3. The percentage inhibition of MDA formation by the different flavonoids and other antioxidants studied is shown in Figure 1. The order of potency of these compounds was found to be: morin > kaempferol > myricetin > quercetin > vitamin A > α-tocopherol > apigenin > (+)-catechin > chrysin > datiscetin > luteolin > naringin > taxifolin > rutin > BHT > naringenin. In contrast, our earlier report (8) using mitochondria as substrate revealed the order of antioxidant potency of the flavonoids to be myricetin (91%), morin (85%), quercetin (78%), (+)-catechin (64%), taxifolin (49%), rutin (39%), apigenin (13%), naringen (10%), chrysin (6%) and naringenin (3%).

DISCUSSION

The flavonols were found to be the best antioxidants (Table 3). However, their potency is affected by the location of the hydroxyl group in the B-ring of the molecule. Hydroxyl substitution in ortho position in the B-ring alone exhibited reduced antioxidative effects as seen with the flavonol and datiscetin. The hydroxyl substitution at the ortho position, when accompanied by additional hydroxyl group at the para position in the B-ring, enhanced the antioxidative action (see morin). Hydroxylation at para position alone in the B-ring also gave rise to a strong antioxidative effect (see kaempferol). Glycosides of flavonoids do not exhibit strong antioxidative action as indicated by rutin and naringin. This suggested that a free hydroxyl substitution at position 3 in C-ring was desirable. In addition, a double bond was required to be present between C-2 and C-3 positions in the C-ring. Taxifolin and naringenin lack such a double bond, and therefore they exhibited low antiperoxidative effect.

The effects of various flavonoids on ethoxycoumarin deethylase activity on rat intestinal and hepatic microsomes were compared by Vernett and Siess (10). They also found that polyhydroxylated flavones and flavonols were more efficient inhibitors than the corresponding flavanones, flavanonols and flavan-3-ols. They suggested

TABLE 2

Analytical Data of RBD-Palm Oil Before and After Refining

	Before (average)	After (average)
1. Free fatty acid	4%	0.05%
2. Peroxide value	3	0
3. Iodine value	50.54	50.54
4. Color	Orange red (visual)	2.5 (>0.25″ cell)
5. Moisture	0.2%	0.02%
6. Trace metals		
—iron	8 ppm (Max)	<1 ppm
—copper	0.2 ppm (Max)	0.01 ppm
7. Tocopherol	600–700 ppm	100.1 ppm
8. Sterol	15 ppm (Max)	4 ppm (Max)
9. Fatty acid profile (wt%)		
$C_{12:0}$	0.2	0.2
$C_{14:0}$	1.1	1.1
$C_{16:0}$	44.0	44.0
$C_{16:1}$	0.1	0.1
$C_{18:0}$	4.5	4.5
$C_{18:1}$	39.2	39.2
$C_{18:2}$	10.0	10.0
$C_{18:3}$	0.4	0.4
$C_{20:0}$	0.4	0.4

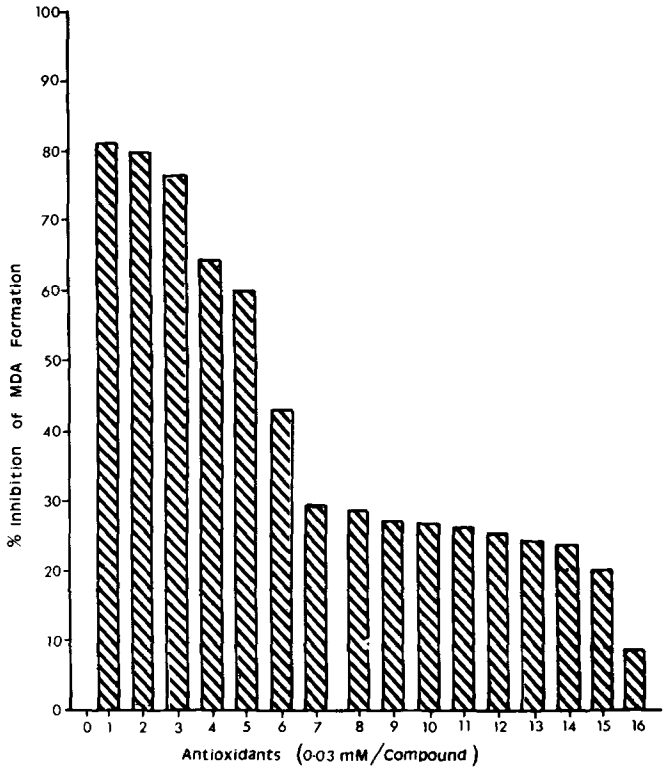


FIG. 1. Percent inhibition of MDA formation by the different flavonoids (0.03 mM/compound) and other antioxidants during thermal autoxidation of refined, bleached and deodorized palm oil. 0, control; 1, morin; 2, kaempferol; 3, myricetin; 4, quercetin; 5, vitamin A; 6, α-tocopherol; 7, apigenin; 8, (+)-catechin; 9, chrysin; 10, datiscetin; 11, luteolin; 12, naringin; 13, taxifolin; 14, rutin; 15, BHT; and 16, naringenin.

TABLE 3

Effects of Flavonoids (0.03 mM/compound) and Other Antioxidants on MDA Production During Thermal Autoxidation of Refined, Bleached and Deodorized Palm Oil (Values are Means of Four Determinations)

	MDA in μmoles ± S.D./g palm oil
Control-palm oil	39.69 ± 0.05
Flavonoids:	
Morin	6.33 ± 0.05
Kaempferol	7.33 ± 0.01
Myricetin	8.53 ± 0.03
Quercetin	13.47 ± 0.09
Apigenin	26.50 ± 0.01
(+)-Catechin	26.68 ± 0.04
Chrysin	27.20 ± 0.06
Datiscetin	28.60 ± 0.01
Luteolin	28.95 ± 0.10
Naringin	29.60 ± 0.08
Taxifolin	29.78 ± 0.05
Rutin	30.01 ± 0.03
Naringenin	36.65 ± 0.30
Other antioxidants:	
Vitamin A	15.23 ± 0.03
α-Tocopherol	21.15 ± 0.04
Butylated Hydroxytoluene (BHT)	30.40 ± 0.05

that the planarity of the flavane nucleus may play a role in determining the intensity of the effect. Moreover, they found that kaempferol was a more powerful inhibitor of MDA production than apigenin and naringenin, suggesting that further hydroxylation at C-3 position in the case of 5,7,4′-OH compounds contribute to the inhibition (10). This was in agreement with our present findings.

Tocopherols are generally present in edible oils and are viewed as natural antioxidants (11). It has long been suggested that α-tocopherol can act simultaneously as a prooxidant and an antioxidant in edible oil (12). In our laboratory, it was reported recently that vitamin A and its analogs, retinal, retinoic acid, retinol acetate and retinol palmitate, were potent antioxidants and might be used as alternative chemicals for the inhibition of lipid peroxidation in animal nutrition (13). In the present report, we have found that vitamin A is a better compound than α-tocopherol as an antioxidant (Table 3).

The polyhydroxylated flavonols morin, kaempferol, myricetin and quercetin exerted stronger inhibitions on the MDA production (Fig. 1) than the better known antioxidants (such as α-tocopherol, vitamin A and BHT) on the thermal autoxidation of RBD-palm oil. Most likely they exert their actions by terminating radical chain reactions and removing molecular oxygen (14). Thus, this present report revealed several flavonoids which may be suitably considered as potential antioxidants for use in the stabilization of palm oil and its fractions during thermal rancidity. The actions of the flavonoids in this study correlate well with those reports of these compounds on the lipid peroxidations in animal tissue or enzyme systems (9,10).

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