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THE EFFECTS OF FLAVONOIDS ON THE THERMAL AUTOXIDATION OF PALM OIL AND OTHER VEGETABLE OILS DETERMINED BY DIFFERENTIAL SCANNING CALORIMETRY

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ABSTRACT

The oxidation times of refined, bleached, deodorised (RBD) palm oil, stabilised by various antioxidants, were measured by differential scanning calorimetry (DSC). Using induction time for the 443 K isotherm, the antioxidant efficiency was found to decrease in the order: myricetin > morin > diphenyl disulphide > quercetin = propyl gallate > kaempferol > retinol > α-tocopherol > ergocalciferol > nordiguaretic acid (NDGA) > butylated hydroxy anisole (BHA). The peak oxidation temperature and the induction time of RBD palm oil, palm olein, palm stearin, palm kernel oil, corn oil, sunflower oil, soyabean oil, olive oil, peanut oil, coconut oil, butter and sunflower margarine were measured using differential scanning calorimetry. From the DSC studies, it was evident that RBD palm oil has a better oxidative stability than olive oil, soyabean and sunflower oils as a frying medium. Determination of the peak oxidation temperature provides a fast reliable method for the detection of adulteration in fats and oils. Myricetin was found to be a good antioxidant for the inhibition of lipid peroxidation when fats and oils are heated at higher temperatures.

INTRODUCTION

Differential scanning calorimetry is a technique for recording the energy necessary to establish zero temperature difference between a substance and a reference material against time or temperature as the two specimens are subjected to an identical temperature regime in an environment heated or cooled at a controlled rate.

Although differential thermal analysis (DTA) and the closely related differential scanning calorimetry (DSC) have been employed for many years in the study of a wide variety of organic and inorganic systems, including polymers, their applications in biochemistry and biology have only recently become important [1]. Sub-ambient DSC has been used to "finger-print"

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vegetable oils commercially selected for their physical properties, price and availability [2]. This method was also used to study lipid phase transitions and drug interactions [3,4]. There are also several reports on the use of DSC to determine the correlation of isothermal induction time, activation energy and oxidation peak temperature of natural and synthetic rubbers [5–8]. However, DSC has not been used to study lipid peroxidation and therefore this report describes the measurement of the induction time of various flavonoids, the peak oxidation temperature of different cooking oils and the activation energy of the reaction. In addition, the antioxidant efficiency of several plant flavonoids and known antioxidants is also examined.

EXPERIMENTAL

Materials

The refined, bleached and deodorised (RBD) palm oil, palm olein, palm stearin and palm kernel oil were donated by Lam Soon Oil and Soap Manufacturing (S) Pte Ltd. The corn oil, coconut oil, olive oil, peanut oil, sunflower oil, butter and sunflower margarine were purchased from a local source.

The flavonoids, kaempferol, morin, myricetin and quercetin, the vitamin A (retinol), vitamin D_3 (ergocalciferol), vitamin E (α-tocopherol), diphenyl disulphide, propyl gallate, nordiguaretic acid (NDGA) and butylated hydroxy anisole (BHA) were purchased from Sigma Chemicals, St. Louis, U.S.A.

Apparatus

A Perkin–Elmer DSC-4 differential scanning calorimeter was used. The instrument was purged with pure oxygen at a flow rate of 20 ml min^{-1} during operation.

Standard antioxidant solutions

Different flavonoid (10 mM) stock solutions were prepared using oxygen-free absolute ethanol as solvent. The working solution was 30 μM for each flavonoid. Similar concentrations (30 μM) were used for other antioxidants, namely vitamin A, α-tocopherol, ergocalciferol, diphenyl disulphide, propyl gallate, nordiguaretic acid (NDGA) and butylated hydroxyanisole (BHA).

The analytical data on RBD palm oil (before and after refining) is given in Table 1.

TABLE 1

Analytical data of RBD palm oil before and after refining

	Before (average)	After (average)
Free fatty acid (%)	4	0.05
Peroxide value	3	0
Iodine value	50.54	50.54
Colour	Orange-red (visual)	2.5 (> 0.25″ cell)
Moisture (%)	0.2	0.02
Trace metals (ppm)		
Iron	8 (max)	<1
Copper	0.2 (max)	0.01
Tocopherol (ppm)	600–700	100.1
Sterol (ppm)	15 (max)	4 (max)
Fatty acid profile (wt.%)		
C12:0	0.2	0.2
C14:0	1.1	1.1
C16:0	44.0	44.0
C16:1	0.1	0.1
C18:0	4.5	4.5
C18:1	39.2	39.2
C18:2	10.0	10.0
C18:3	0.4	0.4
C20:1	0.4	0.4

Sample preparation

Aliquots (2 ml) of palm oil (at 60 °C) were added to different types of flavonoids (30 μM concentration of each of the following compounds: morin, myricetin, kaempferol and quercetin) in separate test tubes. The mixture was vortex-mixed and then cooled to 5 °C (278 K). The other antioxidants, namely vitamin A (retinol), α-tocopherol, ergocalciferol, diphenyl disulphide, propyl gallate, nordiguaretic acid (NDGA) and butylated hydroxyanisole (BHA) (30 μM in 30 μl absolute ethanol for each compound) were similarly added to RBD palm oil.

Measurement of peak oxidation temperature

Frozen RBD palm oil was placed in an empty aluminium cup and another aluminium cup was used as control. These two aluminium cups were uniformly heated at a rate of 20 K min^{-1}. The oxidation exotherm appeared at 489 K and the peak oxidation temperature was located at 573 K (Fig. 1).

Similar experiments were repeated using palm olein, palm stearin, palm kernel oil, corn oil, coconut oil, olive oil, peanut oil, soyabean oil, sunflower oil, butter and sunflower margarine. Peak oxidation temperatures and the

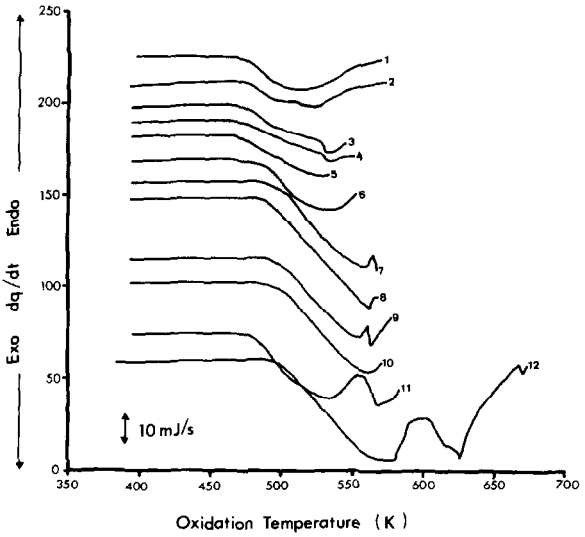

Fig. 1. Peak oxidation temperatures of various oils: 1, peanut oil; 2, corn [mazola] oil; 3, sunflower margarine oil; 4, sunflower oil; 5, soyabean oil; 6, butter oil; 7, palm olein oil; 8, palm kernel oil; 9, palm stearin oil; 10, coconut oil; 11, olive oil; 12, RBD palm oil.

time taken for the oxidation exotherm to appear are presented in Table 2 and in Fig. 1.

Measurement of induction time

RBD palm oil was also heated from 298 K at 20 K min^{-1} up to 443 K. At this final isothermal, the time required for the appearance of the first exotherm was taken as the induction time.

TABLE 2

Peak oxidation temperature and induction time of various oils

Name of oil	Peak oxidation temperature (K)	Induction time (min)
RBD palm oil	573	19.0
Olive oil	568	18.5
Coconut oil	563	17.2
Palm stearin	563	17.2
Palm kernel oil	561	17.0
RBD palm olein	555	16.4
Butter	541	13.8
Soyabean oil	533	14.0
Sunflower oil	533	14.0
Sunflower margarine	531	13.0
Corn (Mazola) oil	523	13.0
Peanut oil	518	12.8

TABLE 3

Induction time of various antioxidants (0.03 μM each dissolved in 30 μl absolute alcohol) added to RBD palm oil (2 ml)

Antioxidants	Induction time (min)	
	453 K (180 °C)	443 K (170 °C)
Control (RBD palm oil)	6.8	12.5 ±0.50
Morin	8.0	16.5 ±1.00
Kaempferol	7.6	15.0 ±2.00
Myricetin	7.5	19.5 ±4.56
Quercetin	7.1	15.4 ±0.90
Vitamin A (retinol)	Not determined	14.35 ±0.30
Vitamin D (ergocalciferol)	Not determined	13.5 ±0.35
Vitamin E (α-tocopherol)	Not determined	14.2 ±0.20
Diphenyl disulphide	Not determined	15.8 ±0.20
Propyl gallate	Not determined	15.4 ±0.30
Nordiguaretic acid (NDGA)	Not determined	13.25 ±0.20
Butylated hydroxy anisole (BHA)	Not determined	13.2 ±0.30

The experiment was repeated using RBD palm oil samples containing 30 μM (dissolved in 30 μl oxygen-free absolute ethanol) of the antioxidants: morin, myricetin, kaempferol, quercetin, vitamin A (retinol), vitamin D_3 ergocalciferol), vitamin E (α-tocopherol), propyl gallate, nordiguaretic acid (NDGA), diphenyl disulphide and butylated hydroxyanisole (BHA), and the respective induction times were determined for the different samples as before and are presented in Table 3.

Measurement of iodine value

Iodine values of various oils and fats were measured using Wij's method [9] and are presented in Table 4.

Table 4

Iodine values of various oils and fats

Oil	Iodine value
Palm oil	50.54
Coconut oil	10.0
Palm kernel oil	22.00
Butter	40.0
Olive oil	90.0
Peanut or ground nut oil	94.0
Corn oil (Mazola oil)	126.0
Soyabean oil	143.0

RESULTS

From Fig. 1 and Table 2, it is clear that palm oil is the best heating medium for frying, having the highest peak oxidation temperature (573 K) compared with other oils and fats. For the lipids studied, the ability to withstand high temperature treatment decreased in the order: palm oil > olive oil > coconut oil > palm stearin > palm kernel oil > palm olein > butter > soyabean oil = sunflower oil > sunflower margarine > corn oil > peanut oil. The logarithm of the induction time t versus the reciprocal of the peak oxidation temperature, T_p (Arrhenius plots) for various samples are shown in Fig. 2. The result revealed a linear relationship between log t and $1/T_p$ for the oil samples and corresponded to the equation

$$\log t = -926\,(1/T_p) + 4.659$$

$$+ \text{ an oxidation activation energy of } 17.7 \text{ kJ mol}^{-1}$$

Based on the induction time presented in Table 3, the effectiveness of the antioxidants studied is in the decreasing order: morin > kaempferol > myricetin > quercetin for an isotherm of 180 °C or 453 K. However, for an isotherm of 170 °C or 443 K, the effectiveness of the antioxidants was altered and showed the decreasing order: myricetin > morin, diphenyl disulphide > quercetin = propyl gallate > kaempferol > vitamin A (retinol) > vitamin E (α-tocopherol) > vitamin D_3 (ergocalciferol) > nordiguaretic acid (NDGA) > butylated hydroxy anisole (BHA) > control RBD palm oil. Thus, the 443 K isotherm was chosen to measure the antioxidant efficiency because it gave a better sensitivity than the 453 K isotherm.

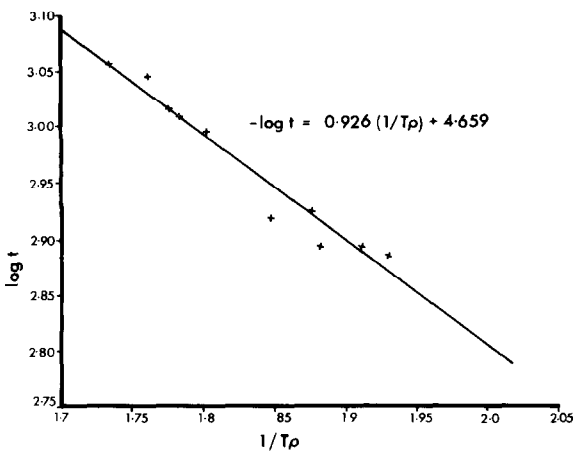

Fig. 2. Arrhenius plot of the logarithm of induction time (t) against the reciprocal of the peak oxidation temperatures (T_p) of various oils.

DISCUSSION

Induction time is a measure of the chain-terminating efficiency of the free radical reaction. It is also a measure of the electron-repelling ability of the antioxidants. Pro-oxidants attract electrons. Bolland and Tenhave [10] have shown that the chain-terminating efficiency, as measured by the length of the induction period of an antioxidant, increases with a decrease in the oxidation–reduction potential (increasing oxidisability). Davies et al. [11] have suggested that electron-repelling substituents increase the rate of the inhibition reaction

$$AH + RO_2^{\cdot} \rightarrow A^{\cdot} + ROOH$$

whereas electron-attracting substituents reduce the antioxidant efficiency. They have also demonstrated the relation between the bond dissociation energy and the antioxidant efficiency: a higher bond dissociation energy means a higher antioxidant efficiency. Cosgrove and Waters [12] compared the oxidation of substituted phenols with benzoyl peroxide and found the rate of oxidation was in the decreasing order: *ortho* > *para* > *meta*. Pereira [13] reported that the antioxidant efficiency of vitamin A oxidation using *t*-butylhydroperoxide could be arranged in the sequence: diphenyl disulphide > propyl gallate > nordiguaretic acid > butylated hydroxy anisole. In our present report, myricetin has been found to be the best antioxidant at the 443 K isotherm, but at 453 K, morin was found to be the best. Reports from this laboratory [14,15] have shown that retinol is better than α-tocopherol and BHT (butylated hydroxytoluene) as an antioxidant.

It has been shown that the partial oxidation products of disulphides, the thiosulphinates (RSOSR), are active inhibitors of free radical reactions [16]. The formation of thiosulphinates would have the two-fold effect of acting as a hydroperoxide destroyer as well as forming a product able to act as an inhibitor of free radical processes.

Among the oils tested, RBD palm oil was found to exhibit better oxidative stability than olive oil, soyabean oil and sunflower oil.

A plot of peak oxidation temperature against iodine value (Fig. 3) shows no inverse correlation (as expected) to indicate that palm oil's resistivity to oxidation does not depend on degree of saturation and it is a good method of 'finger printing' oils and fats and can help to detect the presence of adulterating materials. Peak oxidation temperature will also help in the choice of the best oil or oil blends for frying media in fast-food restaurants and in the baking industry. In the margarine industry, it may help in the choice of oil blends.

This report shows that RBD palm oil is the preferred frying medium for use in the baking and frying industry, rather than olive oil, soyabean oil and sunflower oil. Myricetin, a plant flavonoid, is the best antioxidant that could be used to reduce the peroxidation in fats and oils heated at high tempera-

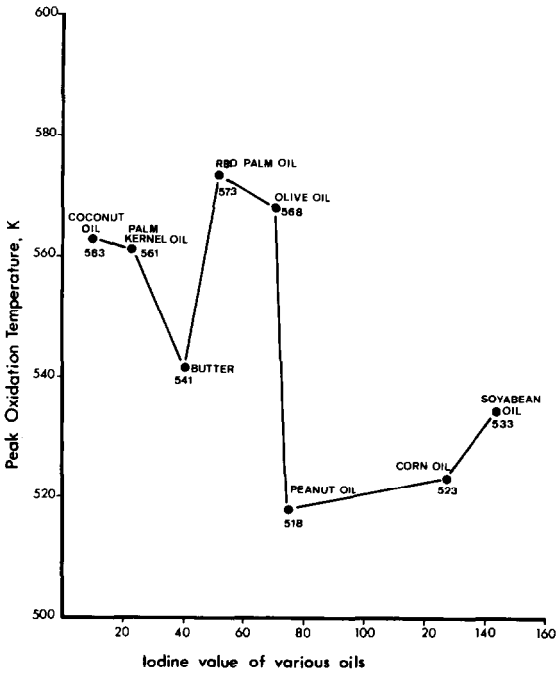

Fig. 3. Effect of peak oxidation temperature on iodine value of some vegetable oils.

tures. It may also be considered as an additive for increasing the shelf-storage life of cooking oils.

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