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Assessment of Thin-Film Oxidation with Ultraviolet Irradiation for Predicting the Oxidative Stability of Edible Oils

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The oxidative stability of edible oils and samples of rapeseed oil with added antioxidants, metal ions, phospholipids and oxidized oil was assessed by a method involving oxidation of a thin film of oil with ultraviolet (UV) irradiation at 100°C. Induction times determined by this method were compared with those determined with the Rancimat at 100°C. The two methods agreed well in describing the effects of additives on the stability of the edible oil. Induction times were considerably shorter for the thin-film UV method, and the method may have potential as an accelerated test method for assessing the effect of additives on the oxidative stability of relatively stable oils and fats. The correlation between the Rancimat and the thin-film UV induction times also was assessed at 80°C for rapeseed oil containing additives, but there was no advantage in using the lower temperature alone because the induction times were 2–7 times longer than at 100°C. However, use of two elevated temperatures is likely to improve predictions of stability at lower temperatures, especially for samples containing copper, which have an exceptionally high-temperature coefficient. The thin-film UV method showed a poorer agreement with the Rancimat for comparing the oxidative stability of some fats and oils. For instance, corn oil was more stable than soybean oil in the Rancimat test but the order of stability was reversed in the thin-film UV test. Cocoa butter was much more stable in the Rancimat test than when assessed by the thin-film UV test.

KEY WORDS: Film, oxidation, stability.

The resistance of edible oils to oxidative deterioration, leading to rancid off-flavors, is an important parameter for assessing the quality of an oil and its suitability for use in foods or food processes. To predict the oxidative stability of edible oils, several accelerated tests have been developed. These tests most commonly rely on accelerating deterioration by the use of heat, as in the Schaal oven test (1), or a combination of heat and bubbling air though the sample, as the Active Oxygen Method (2) or the Rancimat Method (3). Other methods of accelerating deterioration include addition of metal ions (4) or the action of light (5), with short-wave ultraviolet (UV) radiation being most effective (6). It is also well recognized that oxidation is most rapid for samples with high surface area in contact with air (7). The Rancimat test is commonly used for assessing the oxidative stability of oils and fats, but induction times (ITs) at 100°C can be in excess of 230 h for relatively stable fats such as cocoa butter (8). The use of temperatures higher than 50°C to predict room-temperature stability has been criticized because the rate-limiting steps may change (9). Even at lower temperatures, the relative rates of lipid oxidation steps are dependent on the presence of minor components, such as metal ions, citric acid or ascorbic acid (10). However, there is a need for effective acceleration of oxidative deterioration in relatively stable fats, and this paper reports the use of a combination of thin-film storage at 80 and 100°C with UV irradiation as an efficient method of accelerating oxidative deterioration. Although sensory assessment of off-flavor development is the procedure most relevant to oil shelf life, instrumental methods for determining induction times were used in this study to allow investigation of a wide range of samples.

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MATERIALS AND METHODS

Refined, bleached and deodorized rapeseed oil was supplied by PURA Foods Ltd. (London, England). Sunflower, safflower, soybean, corn and refined olive oils were purchased from Asda Superstore (Reading, England), and cocoa butter was supplied by Leatherhead Food RA (Leatherhead, England). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were purchased from Koch Light Laboratories (Colnbrook, England) and tertiary butyl hydroquinone (TBHQ) was purchased from Fluka Chemicals Ltd. (Gillingham, England). Citric acid was purchased from Fisons Scientific Equipment (Loughborough, England).

Ferric palmitate and cupric stearate, prepared as described previously (8,11) were used for addition of ferric and cupric ions. Addition of antioxidants to rapeseed oil was achieved by dissolving the additive (500 mg) in oil (100 g) by heating to 50°C with stirring until the antioxidant was dissolved (0–30 min). The sample was then diluted to the required concentration. A similar procedure was followed for addition of cupric stearate and ferric palmitate, but heating to 60°C was used. Addition of phospholipids to rapeseed oil was achieved by adding 100 mg to the oil before dilution, and for phosphatidylcholine, heating to 70°C was required.

Citric acid, when required, was added as an aqueous solution (3 mL, 1.3%), added to rapeseed oil (1000 g) with stirring at 50°C. The water was removed by evaporation under vacuum (10 torr) at 60°C, before dilution with rapeseed oil to the required concentration.

Rancimat oxidation experiments were performed by using a Metrohm Rancimat Model 617 (V.A. Howe & Co., Ltd., Banbury, United Kingdom) with 2.5 g oil and an air flow rate of 15 L per h. Glass vessels were cleaned by heating in boiling sodium hydroxide solution (2% m/m) for 1 h, soaking in concentrated hydrochloric acid and washing with distilled water. All determinations were performed in duplicate, and the results were averaged.

Partially oxidized rapeseed oil was prepared by oxidizing rapeseed oil (15 g) in four glass tubes in the Metrohm Rancimat at 100°C. The samples were removed before the end of the induction period, and the peroxide value of the combined oxidized oil sample was 75 meq per kg. The sample was used as an additive to fresh rapeseed oil at levels of 5, 10 and 50%.

Oxidation of thin films was performed in duplicate in porcelain crucibles (5 cm diameter, 1 cm deep), supported in an aluminum block placed in an oil bath at either 80 or 100°C. The oil sample (2.5 g) was weighed into the crucible and irradiated with a six-watt short-wave UV lamp (200–280 nm), placed 3 cm above the surface of the oil. A sample (200 mg) was removed periodically for analysis. Induction times were determined by plotting the peroxide value, the headspace volatile peak areas and the UV absorbance at 232 nm against time and determining the time before the gradient of each plot showed a marked increase.

The peroxide value of oil samples was determined by the colorimetric micromethod as described by Asakawa and Matsushita (12). This method involved reaction with potassium iodide and aluminum chloride prior to color development with starch. Absorbance was determined at 560 nm with a Perkin-Elmer Lambda 5 spectrophotometer (Palo Alto, CA).

Oil deterioration also was assessed by the gas chromatographic static headspace analysis of volatiles by a method based on that of Marsili (13). The oil sample (100 mg) was weighed into a pressure-resistant vial (6 mL), which was sealed and purged with nitrogen for 3 min. The vials were placed in the magazine of a Perkin-Elmer HS-6 headspace sampler, which was preheated to 180°C. The vials were held at 180°C for 1 h before analysis in order to ensure complete decomposition of hydroperoxides. At the end of this time, the headspace was injected and analyzed with a BP5 capillary column (25 m × 0.25 mm) in a Perkin-Elmer Sigma 3B gas chromatograph. The oven was cooled with liquid carbon dioxide and programmed from −20°C (5 min) rising to 130°C at 10°C min^{-1}, followed by 2 min at 130°C. Chromatographs were recorded and peaks integrated by a Hewlett-Packard 3390 integrator (Palo Alto, CA).

Oil oxidation also was assessed by the absorbance at 232 nm of solutions of oil (40–80 mg) in hexane (100 mL). The absorbance was determined by using a Perkin-Elmer Lambda 5 spectrophotometer.

Copper and iron were determined by burning rapeseed oil (10 g) in a silica crucible over a Bunsen burner. The residue was heated in a muffle furnace at 850°C for 1.5 h. The residue was dissolved in nitric acid (5 mL, 6.0 M), and metal concentrations were determined by atomic absorption spectrophotometry at 324.8 nm for copper and 248.4 nm for iron with a Pye Unicam SP9 spectrophotometer (Unicam Analytical Systems, Cambridge, United Kingdom). An external standard method was employed for the analysis with standards in the range of 0.5–2.5 ppm prepared by dissolving copper or iron (1 g) in nitric acid (100 mL, 6.0 M), followed by dilution to the required concentration.

Fatty acid methyl esters were prepared from oils by a sodium methoxide-catalyzed transesterification method described by Christie (14). Analysis of fatty acid methyl esters was performed with a packed column (5 m × 3 mm i.d.), containing Chromosorb W coated with 15% diethylene glycol succinate at 180°C. A Hewlett-Packard 3390 integrator was used to record and quantitate the chromatogram. Regression analysis of the data was performed with a Minitab program (15).

RESULTS AND DISCUSSION

Thin-film oxidation under UV irradiation at 100°C was studied as an alternative oxidation procedure to the Rancimat to investigate whether it could be used as an accelerated test method for relatively stable fats that have long induction times in the Rancimat. Initially, a sample of refined rapeseed oil was oxidized by the thin-film UV method, which showed a large increase, after oxidation for 2 h, in the total volatile peak area assessed by gas chromatographic headspace analysis, in the absorbance of a sample at 232 nm and in the peroxide value (Table 1). Consequently, these three methods were selected for determining the IT for samples oxidized by the thin-film UV method.

The catalytic effect of UV irradiation during oxidation at 100°C was confirmed by measurement of UV absorbance and headspace analysis on thin films held at 100°C in the presence and absence of UV irradiation. UV absorbance had increased 3.9 times in 4 h with irradiation, compared with 1.1 times in the dark and the volatile concentration had increased 28 times with irradiation compared with 1.4 times in the dark (Table 2).

The oxidative stability of 31 samples, consisting of 7 fats and 24 samples of rapeseed oil with additives, was assessed by the Rancimat and by thin-film oxidation with UV irradiation, both assessments being performed in duplicate at 100°C (Table 3), and the results were averaged. A temperature of 100°C was chosen to give reasonably short ITs for a wide range of samples, including oils containing antioxidants. The determination of ITs at 80°C also was investigated (Table 4), but the ITs were 2–6 times longer than at 100°C for the Rancimat and 3–7 times longer for the thin-film method. The relative change in IT for volatile antioxidants, such as BHT, was not significantly greater than for nonvolatile additives, such as α-tocopherol, in both the Rancimat and the thin-film UV tests. Therefore, the reported inappropriateness of the Rancimat method at 100°C for volatile antioxidants (16) was not overcome either by performing the Rancimat test at 80°C, or by using the thin-film UV method at 80 or 100°C.

The advantage of using measurement from at least two elevated temperatures to predict room temperature stability, as recommended by Ragnarsson and Labuza (9), was evident for samples containing copper because the temperature coefficient was particularly high for these samples. The ratios of ITs of samples, containing copper and measured at 80 and 100°C (IT_{80}/IT_{100}), was 5.0–5.4 in the Rancimat and 6.1–6.8 by oxidation in thin films. This compares with ratios of 2.3–4.7 and 3.6–4.9, respectively, for samples that do not contain metal ions.

TABLE 1

Changes in Total Volatiles by Gas Chromatography (GC) Headspace Analysis, Peroxide Value and Conjugated Dienes for a Rapeseed Oil Sample Oxidized by the Thin-Film Ultraviolet (UV) Method

	Time		
	0 h	2 h	4 h
GC-headspace volatiles peak area (× 10^6 integrator counts)	0.73	1.64	16.55
Peroxide value (meq/kg)	9.1	27.3	98.2
UV absorbance	1.55	1.82	6.13

TABLE 2

Effect of UV Radiation on Rapeseed Oil Deterioration in a Thin Film at 100°C[a]

	GC Headspace volatile peak area [× 10^6 integrator counts)]		UV Absorbance (at 232 nm)	
Time (h)	UV illumination	Dark	UV illumination	Dark
0	0.60	0.60	1.57	1.57
2	1.67	0.60	1.82	1.56
4	16.53	0.82	6.06	1.66

[a]See Table 1 for abbreviations.

TABLE 3

Induction Times of 31 Samples Assessed by the Rancimat and by Thin-Film Oxidation Under UV Irradiation at 100°C

	Thin-film UV induction times (h)				Rancimat induction time[a] (h)
Sample	PV[a]	UV[a]	GC[a]	Mean[b]	
Fats					
Rapeseed oil (RO)	6.1	5.3	6.5	6.0 (0.6)	14.4
Olive oil	5.1	—	6.8	6.0 (1.0)	19.9
Corn oil	2.0	2.2	2.6	2.3 (0.4)	12.8
Soybean oil	3.7	3.1	4.2	3.7 (0.5)	10.9
Sunflower oil	1.7	1.5	2.1	1.8 (0.4)	7.9
Safflower oil	1.7	2.1	2.1	2.0 (0.2)	6.8
Cocoa butter	47.3	48.0	48.8	48.0 (1.0)	230.3
RO and additives					
α-Tocopherol (0.02%)	5.5	6.4	4.5	5.5 (0.9)	11.2
TBHQ (0.02%)	44.0	44.0	44.0	44.0 (0.0)	94.8
BHT (0.02%)	9.0	9.0	9.0	9.0 (0.8)	20.5
BHA (0.02%)	8.4	8.0	9.6	9.0 (0.9)	19.3
BHT (0.01%) + BHA (0.01%)	8.9	9.3	9.2	9.1 (0.9)	20.3
Phosphatidylethanolamine (0.1%)	7.4	8.1	8.7	8.1 (0.9)	22.8
Lecithin (0.1%)	5.8	6.3	6.8	6.3 (0.9)	18.2
Phosphatidylinositol (0.1%)	6.0	6.4	6.3	6.2 (0.8)	15.5
Phosphatidylcholine (0.1%)	5.4	5.7	6.9	6.0 (0.9)	14.0
Citric acid (0.02%)	6.7	5.8	6.3	6.3 (0.9)	17.3
Citric acid (0.01%)	6.1	7.7	6.9	6.9 (0.9)	16.9
Citric acid (0.02%) + Fe^{3+} (0.14 ppm)	5.6	6.7	6.9	6.4 (0.8)	16.6
Citric acid (0.01%) + Fe^{3+} (0.14 ppm)	6.9	6.6	6.8	6.8 (0.8)	14.4
Citric acid (0.02%) + Cu^{2+} (0.1 ppm)	4.8	5.5	6.4	5.6 (0.9)	14.1
Citric acid (0.01%) + Cu^{2+} (0.1 ppm)	5.5	5.8	6.9	6.1 (0.8)	13.3
Fe^{3+} (0.07 ppm)	5.9	5.3	5.6	5.6 (0.6)	13.6
Fe^{3+} (0.14 ppm)	4.3	3.5	5.1	4.3 (0.7)	10.0
Fe^{3+} (0.35 ppm)	3.0	2.4	3.5	3.0 (0.5)	8.7
Cu^{2+} (0.05 ppm)	3.2	2.8	3.0	3.0 (0.3)	6.3
Cu^{2+} (0.10 pm)	2.1	1.7	2.5	2.1 (0.4)	5.0
Cu^{2+} (0.25 ppm)	1.8	1.2	1.4	1.5 (0.3)	2.8
Oxidized RO (5%)	4.4	4.0	4.2	4.1 (0.5)	13.6
Oxidized RO (10%)	3.7	3.7	4.5	4.0 (0.5)	11.7
Oxidized RO (50%)	3.1	3.1	3.1	3.1 (0.5)	7.6
CV (%)	9.7	13.9	9.4		6.1

[a]Mean of two determinations. PV, peroxide value; BHT, butylated hydroxytoluene; BHA, butylated hydroxanisole; TBHQ, tertiary butyl hydroquinone; CV, coefficient of variation. See Table 1 for other abbreviations.
[b]Mean and (standard deviation) for six determinations.

The IT for oxidation under the thin-film UV conditions was shorter than the Rancimat IT for all samples. The rapeseed oil used for this study had a copper content of 0.015 ppm and an iron content of 0.2 ppm.

A good linear correlation existed between the ITs assessed by the thin-film oxidation method (I_{TF}) and those assessed by the Rancimat (I_R) at 100°C (Fig. 1). The regression equation was:

$$I_{TF} = 0.47\ I_R - 1.11 \quad [1]$$

with a correlation coefficient of r = 0.99 ($P < 0.001$, if all samples excluding the cocoa butter sample were included).

At 80°C (Fig. 2), the regression equation was:

$$I_{TF} = 0.52\ I_R - 0.34\ (r = 0.99;\ P < 0.001) \quad [2]$$

The cocoa butter sample had a much long IT than all other samples when assessed by the Rancimat, but the extent of the increased stability of cocoa butter was not reflected in the increase in the IT when assessed by the thin-film UV method. For example, cocoa butter and the sample of rapeseed oil containing TBHQ had ITs at 100°C

TABLE 4

Induction Times of 25 Samples of Rapeseed Oil Containing Additives Assessed by the Rancimat and by Thin-Film Oxidation Under UV Irradiation at 80°C[a]

Additive	Thin-film UV induction times (h)			Rancimat induction time (h)
	PV	UV	Mean	
—	24.0	24.0	24.0	48.3
α-Tocopherol (0.02%)	20.0	20.0	20.0	41.3
TBHQ (0.02%)	216.0	216.0	216.0	416.5
BHT (0.02%)	38.4	39.7	39.0	70.0
BHA (0.02%)	35.4	34.9	35.1	65.5
BHT (0.01%) + BHA (0.01%)	35.8	35.8	35.8	70.5
Phosphatidylethanolamine (0.1%)	33.9	34.4	34.1	52.6
Lecithin (0.1%)	26.9	26.9	26.9	68.9
Phosphatidylinositol (0.1%)	26.0	26.4	26.2	58.4
Phosphatidylcholine (0.1%)	24.2	25.7	24.9	53.3
Citric acid (0.02%)	27.3	29.0	28.1	70.7
Citric acid (0.01%)	30.2	30.2	30.2	70.9
Citric acid (0.02%) + Fe (0.14 ppm)	28.3	27.6	27.9	70.1
Citric acid (0.01%) + Fe (0.14 ppm)	33.1	32.5	32.8	69.5
Citric acid (0.02%) + Cu (0.1 ppm)	31.0	32.4	31.7	72.8
Citric acid (0.01%) + Cu (0.1 ppm)	33.2	34.8	34.0	68.1
Fe (0.07 ppm)	22.4	23.9	23.1	48.0
Fe (0.14 ppm)	17.2	16.6	16.9	47.0
Fe (0.35 ppm)	12.0	12.0	12.0	42.5
Cu (0.05 ppm)	17.7	18.9	18.3	33.5
Cu (0.10 ppm)	13.9	14.6	14.2	25.0
Cu (0.25 ppm)	9.1	10.7	9.9	15.3
Oxidized RO (5%)	17.4	19.1	18.2	46.5
Oxidized RO (10%)	16.2	15.7	15.9	44.1
Oxidized RO (50%)	11.9	11.9	11.9	35.5
CV (%)	3.6	4.7		2.8

[a]See Tables 1 and 3 for abbreviations.

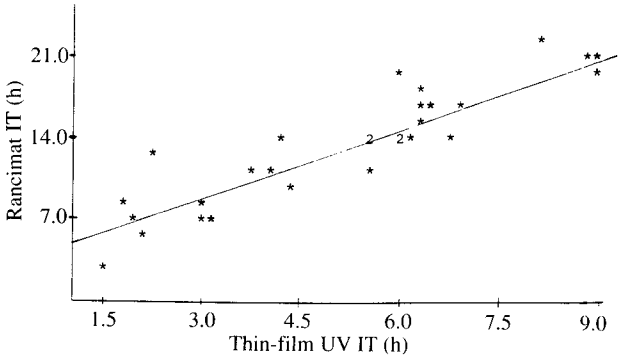

FIG. 1. Plot of Rancimat induction time (IT) vs. thin-film ultraviolet (UV) IT for oxidation of 29 samples at 100°C. The rapeseed oil containing tertiary butyl hydroquinone and the cocoa butter samples are omitted to avoid compressing the plot of the other samples.

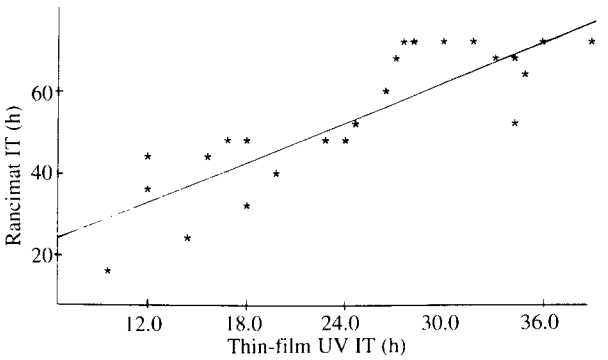

FIG. 2. Plot of Rancimat IT vs. thin-film UV IT for oxidation of 24 samples at 80°C. The rapeseed oil containing tertiary butyl hydroquinone is omitted to avoid compressing the plot of the other samples. See Figure 1 for abbreviations.

of 230.3 h and 94.8 h, respectively, when assessed by the Rancimat. But the values were 48.0 and 44.0 h, respectively, when the two samples were assessed by the thin-film UV method. The different behavior of the cocoa butter sample compared to all other samples may have arisen from the presence of minor components that were removed during the refining of oil. It was the only unrefined fat in the study, and it is known that minor components, e.g., carotenes and chlorophyll, modify the oxidation mechanism in the presence of UV light. Alternatively, a prooxidant may be swept from the fat by the air in the Rancimat test during the long induction period. It should also be noted that cocoa butter has a very different fatty acid composition from the other oils used in this study (Table 5).

The effects of additives were similar in order for both the thin-film and Rancimat tests (Tables 3 and 4). Thus, the order of stability for samples of rapeseed oil containing antioxidants was TBHQ > BHT ~ BHA > α-tocopherol. The order of stability for rapeseed oil containing metal ions showed the progressive reduction in stability of samples with increased concentrations of iron or copper,

TABLE 5

Fatty Acid Composition of Oils (area %)

	Fatty acid				
Oil	16:0	18:0	18:1	18:2	18:3
Olive oil	10.4	2.5	75.8	9.6	0.9
Rapeseed oil	5.3	1.7	60.9	21.1	10.2
Corn oil	9.9	1.9	29.5	57.1	10.2
Soybean oil	11.0	3.4	24.0	54.2	7.1
Sunflower oil	6.6	4.5	19.0	65.8	0.6
Safflower oil	7.1	2.7	13.7	75.9	0.3
Cocoa butter	25.9	36.6	33.1	2.7	1.3

with copper being more detrimental than iron. The stabilizing effect of citric acid also was evident in these samples. The stability of rapeseed oil was reduced by additions of oxidized oil, and phosphatidylethanolamine was more effective than other phospholipids in improving the stability of rapeseed oil.

The difference in stability of different oils agreed less well when studied by the two methods, with the Rancimat test giving the order of stability as cocoa butter > > olive oil > rapeseed oil > corn oil > soybean oil > sunflower oil > safflower oil. This compares with the order cocoa butter > > rapeseed oil ~ olive oil > soybean oil > corn oil ~ safflower oil ~ sunflower oil when the samples were assessed by thin-film UV method. The relative effects of different fatty acids or minor components on oxidative stability are likely to affect the two test methods to different extents. Alternatively, the constantly bubbling air stream in the Rancimat test may remove volatile components that influence the course of the oxidation as well as continuously saturating the sample with oxygen.

The reproducibility of the IT was poorer for samples oxidized by the thin-film UV method, with coefficients of variation of 0–21% being observed in the present study, whereas the Rancimat method is capable of yielding coefficients of variation of <3% (11).

It therefore appears that the thin-film UV method may be useful for studies of the effect of additives on the oxidative stability of relatively stable oils, because for these samples the more rapid assessment of the stability by the thin-film UV method would be advantageous. However, the method may be less suitable for comparing the stability of fats that vary widely in fatty acid composition because it is known that the Rancimat method is successful in predicting the stability under room temperature storage for a wide range of oils and fats (17). This study suggests the possibility of developing an automated instrument for accelerated testing of oil stability. UV irradiation and UV absorption may be combined in a single unit to catalyze deterioration of the oil and measure oil stability. Control of film thickness to give suitable absorption and access to air would be required.

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[Received December 27, 1993; accepted September 8, 1994]