Determination of the Oxidative Stability of Vegetable Oils by Rancimat and Conductivity and Chemiluminescence Measurements

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ABSTRACT: The oxidative stability of five different oils was determined by Rancimat analysis with conductivity and chemiluminescence measurements for evaluation of the induction periods. Samples of oil, taken at intervals from the Rancimat apparatus, were used for chemiluminescence measurements. The chemiluminescence results were plotted vs. time, and the resulting curves were evaluated with a graphical tangential procedure in the same way as the curves of the Rancimat method (conductivity measurement). Induction periods of the oils assessed by Rancimat and chemiluminescence methods showed a significant linear correlation ($r = 0.9865$). The temperature dependence of the induction periods evaluated by chemiluminescence and by conductivity was investigated with walnut oil. A marked temperature dependence was observed for both.
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KEY WORDS: Chemiluminescence, conductivity, induction period, oxidative stability, Rancimat, rapeseed oil, safflower oil, sunflower oil, vegetable oils, walnut oil.

A number of methods for assessment of the oxidative stability of edible fats and oils have been described in the literature (1–4). Two well-known methods for measuring oxidative stability are the active oxygen method (AOM) (1) and the Rancimat method (5,6). In the former method, an oil is heated at 100°C, and the oxidation progress is followed by measuring the peroxide values of heated samples at regular time intervals until a peroxide value of 100 is reached, which gives the AOM endpoint. The Rancimat method, developed by Hadorn and Zürcher (2), is based on the fact that the volatile acids formed during oxidation (7,8) can be used for an automated endpoint detection. In both methods, the measurement parameters, peroxide value, and volatile acids formation show a considerable increase at the beginning of the oxidation phase, so that the endpoint of the induction period (IP) is characterized.

Both methods differ from the use of normal storage conditions by using a flow of air through the sample, held at high temperature to accelerate oxidation of the sample. Mainly hydroperoxides are formed under ambient storage conditions. These hydroperoxides slowly break down to give volatile products. Under accelerated oxidation conditions (such as in the AOM and the Rancimat methods), the rate of formation of hydroperoxides and their breakdown into volatile products are expected to take place simultaneously (2). Gordon and Mursi (9) showed that the Rancimat method at 100°C correlated highly with oil stability as measured by peroxide development during storage at 20°C. A collaborative study of the Rancimat method was described by Jebe *et al.* (10). In that study, the advantages of the Rancimat method and its usefulness at higher temperatures were pointed out.

Another parameter for describing the oxidative status of an oil is the integral of the light curve measured during the chemiluminescence reaction (11). In this method, hydroperoxides formed during the first step of autoxidation, other peroxides, and radicals react with luminol in the presence of a catalyst, such as hemin. The produced light is detected by a photomultiplier, and the resulting light curves are established by integration of the area below the curves. The chemiluminescence method is an exceptionally sensitive method for the measurement of lipid oxidation. This method is also advisable for highly oxidized oils, provided that appropriate measurement parameters are used (11). Matthäus *et al.* (12) described a linear correlation ($r = 0.993$) between the results of the iodimetric peroxide determination (13) and the chemiluminescence method for a model system that consisted of linoleic acid methyl ester and tricaprylin. For commercial oils, there is an exponential correlation between the results of the chemiluminescence method and the iodimetric peroxide determination (12). The differences in sensitivity of the chemiluminescence reaction to various types of hydroperoxides (14) and naturally present tocopherols (15), normally found in commercial oils and fats, have been reported in the literature.

In contrast to the iodimetric method for peroxide determination, the chemiluminescence method also can be used to detect radical compounds [such as alkoxyl-(RO$^\bullet$), peroxyl-(ROO$^\bullet$) or hydroxyl-(HO$^\bullet$) radicals] besides peroxides (16). The chemiluminescence method detects peroxyesters and dialkylperoxides as well (15), whereas the iodimetric method is

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not suitable to determine these compounds because of their high redox potential (17).

As shown by Hadorn and Zürcher (2), who used the Rancimat method, peroxide formation, development of formic acid, and the shift of double bonds show a high degree of linear correlation with each other at 110°C. As yet, no results have been published about the correlation between Rancimat conductivity and chemiluminescence measurements. The objective of this paper is to draw a comparison between the Rancimat method and the chemiluminescence measurement as a means for assessing the oxidative stability of different oils.

MATERIALS AND METHODS

Materials. Samples of walnut, safflower, sunflower, and two rapeseed oils were procured from a local supermarket. The Model 617 Rancimat (Metrohm AG, Herisau, Switzerland) was used as previously described (6,18). Air flow rate was set at 20 L/h, and temperature of the heating block was set at 120°C as recommended by Metrohm (5). The temperature of the conductivity tube was constant at 21°C, the same as the temperature of the reaction vessel of the chemiluminescence measurement.

Effect of temperature on IP. The temperature dependence of both methods was investigated at 100, 110, 120, and 130°C. The IP of each of the five samples were determined in quadruplicate with the Rancimat method, and the mean value is reported. The sample (20 g) was weighed into the reaction vessel. During the Rancimat measurement, 2-g samples were withdrawn at suitable intervals of time and used for the chemiluminescence measurements as described previously (11). In this method, 0.50 g of the sample (in quadruplicate) was dissolved in 5 mL of acetone/ethanol (2:1) solvent mixture. One mL of a luminol solution (consisting of 0.7 mM luminol, 3.85 µM hemin, and 11.8 mM sodium carbonate in double-destillated water) was added to 1 mL of the acetone/ethanol solution. A photomultiplier below the sample vessel received the light and converted it to an electric pulse, whose amplitude was proportional to the intensity of the received light. Each sample taken from the Rancimat was measured four times by the chemiluminescence method, and the mean values were calculated.

Curves obtained by the Rancimat method were evaluated by a graphical tangential method (6,18) to calculate the IP. The area values measured by the chemiluminescence method were plotted vs. time, and the resulting curves were also evaluated graphically.

Effect of sample withdrawal from the reaction vessel on IP. The IP of two of the oils were determined with and without withdrawing 2-g samples to check if any effect could be observed on the IP as a result of periodically withdrawing 2-g samples of oil from the reaction vessel during the Rancimat measurement. Four replications were run with each sample.

The fatty acid and tocopherol compositions of the oils were determined according to Schulte and Weber (19) and Balz *et al.* (20) by means of gas–liquid chromatography and high-performance liquid chromatography procedures, respectively. The iodine values of the different oils were calculated directly from fatty acid compositions according to AOCS method Cd 1c-85 (21).

Data were analyzed by analysis of standard deviation and regression, and the statistical significance of the regression was tested by Kendall's Tau test (22). The Student's t-test to evaluate the statistical significance for independent and variable interactions was performed with two-tailed t-tests at $P = 0.005$. The data were evaluated by a computer program (23).

RESULTS AND DISCUSSION

Effect of withdrawal of sample from the reaction vessel on IP. The IP of walnut oil and rapeseed oil 1, determined with the Rancimat method at 120°C with and without withdrawing 2-g samples during the measurement, are given in Table 1. As indicated, no statistically significant effect ($P < 0.005$) was observed by withdrawing 2-g samples from the reaction vessel. This result is in good agreement with the determinations of Hadorn and Zürcher (2), who also found no influence of the sample size of oil (2.5, 5, and 10 g) on the IP determined by the Rancimat method. However, Hill and Perkins (24) found a positive effect (decrease in IP with increase of sample size) on the IP for sample sizes of 2.5 and 5 g oil.

IP of oils by Rancimat conductivity and chemiluminescence methods. The fatty acid composition and calculated iodine value of the five oils studied is presented in Table 2, and the tocopherol composition has been given in Table 3. The IP of walnut, safflower, sunflower, and two rapeseed oils were determined by the Rancimat method with conductivity and chemiluminescence measurements. Graphical evaluation of the curves obtained with the Rancimat method shows different IP (Table 4), which are negatively correlated with iodine values ($r = -0.9255$). The total tocopherol content also showed a high correlation with IP ($r = 0.9848$), although no correlation between the separate tocopherols (α- to δ-tocopherol) was observed with the IP of the oils ($r = -0.3456$–0.3965). The correlation between the iodine value and the total tocopherol content vs. IP was statistically signif-

TABLE 1
Comparison Between the Induction Periods (IP) of Walnut Oil and Rapeseed Oil 1 with and without Removal of Two-Gram Samples During the Rancimat IP Measurement

	Walnut oil		Rapeseed oil 1	
	Sampling	No sampling	Sampling	No sampling
Number of samples	4	4	4	4
Average IP[a] (min)	80	84	206	209
SD (min)	3.69	3.74	4.13	5.15
Computed t statistic[b]	1.331		1.539	
Significance level[b]	0.232		0.241	
Variance (%)	6.7	5.9	7.8	8.4

[a] Average of the IP of quadruplicate runs.
[b] Results of the statistical hypothesis tests for hypothesis $H_0 = 0$.

TABLE 2
Fatty Acid Composition and Calculated Iodine Value of the Studied Oils

Oils	Fatty acid composition (rel %)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	IV[a]
Walnut	7.3	2.6	18.6	58.4	12.3	149
Safflower	4.6	1.8	20.8	59.5	9.9	147
Sunflower	6.1	5.1	20.7	66.2	0.1	133
Rapeseed 1	5.0	1.7	59.7	21.4	9.6	114
Rapeseed 2	4.7	1.7	59.4	20.9	10.0	113

[a] Calculated iodine value (IV) (Ref. 21).

TABLE 3
Tocopherol Content (mg/kg) of the Studied Vegetable Oils

Oil	Tocopherols (mg/kg)				
	α-T	β-T	γ-T	δ-T	Total
Walnut	7.25	8.0	375.8	36.4	492.7
Safflower	567.2	16.5	18.9	5.3	607.9
Sunflower	604.5	22.2	5.9	—	632.6
Rapeseed 1	212.8	3.2	352.1	91.8	659.9
Rapeseed 2	263.7	2.6	428.5	16.4	711.2

TABLE 4
Iodine Value, Tocopherol Content, and Oxidative Stability of the Studied Vegetable Oils at 120°C

Oils	IV[a]	Total tocopherols[b]	Oxidative stability[c]			
			Chemiluminescence (min)[c]		Rancimat (min)[c]	
Walnut	149	492.7	75	3.97[d]	84	3.69[d]
Safflower	147	607.9	135	12.3[d]	143	3.56[d]
Sunflower	133	632.6	165	9.57[d]	155	3.24[d]
Rapeseed 1	114	659.9	197	14.5[d]	209	4.13[d]
Rapeseed 2	113	711.2	232	19.5[d]	224	2.15[d]

[a] Calculated iodine value (IV) (Ref. 21).
[b] Sum of α-, β-, γ-, and δ-tocopherols (mg/kg).
[c] Mean of four determinations.
[d] SD (min).

icant according the Kendall's Tau test. No statistical significance was observed for the other parameters (e.g., 18:1 vs. IP; 18:2 vs. IP or α-tocopherol vs. IP, etc.).

The resulting curves of the chemiluminescence method (Fig. 1) show similar IP (Table 4). There was a statistically significant correlation ($r = 0.9865$) (Fig. 2A) between the IP assessed by the Rancimat (conductivity) and the chemiluminescence methods for the five oils at 120°C. No significant difference was recognizable between the mean values of the IP of both methods ($P < 0.005$). The variation of the measurements ranged between 4 and 11%, depending on the amount of peroxides and radicals in the reaction vessel of the chemiluminescence measurement (15).

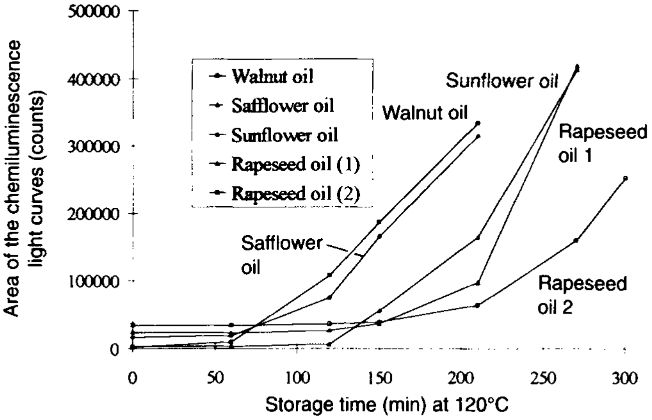

FIG. 1. Stability curves for different edible oils oxidized at 120°C in the Rancimat as measured by the chemiluminescence method.

This shows that both methods lead to the same result at a temperature of 120°C for the five oil samples, although the chemiluminescence measurements detected only primary products, and the conductivity measurement of the Rancimat method detected stable secondary products of the autoxidation. This observation also indicates that the formation of hydroperoxides, peroxides, and radicals (determined by taking the light curves of the chemiluminescence reaction), and the development of volatile compounds (determined by measurement of the conductivity with the Rancimat method) occur si-

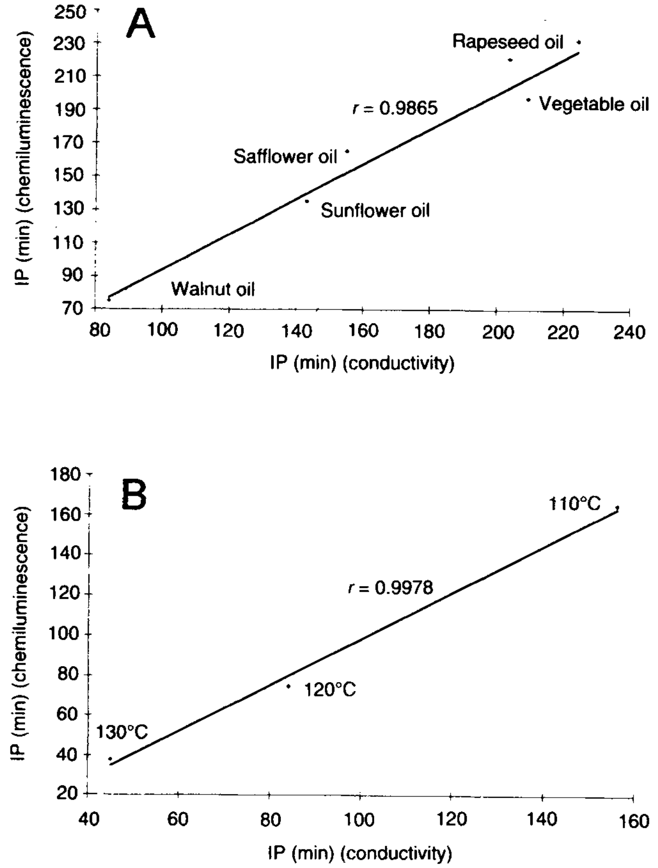

FIG. 2. A. Correlation of the induction periods (IP) of different oils evaluated by measurements of conductivity and chemiluminescence measurements at 120°C. B. Correlation of the IP of walnut oil measured by the two methods at different temperatures.

TABLE 5
Effect of α- and δ-Tocopherol on the Area of the Chemiluminescence Light Curves (Ref. 15)

α-Tocopherol		δ-Tocopherol	
Conc. (mg/kg fat)	Area[a]	Conc. (mg/kg fat)	Area[a]
241	147884	101	288284
990	320674	998	367842
1990	108061	2001	985821

[a] Difference between the area of the light curves of methyl linoleate/tricaprylin model system with and without addition of tocopherol. Conc., concentration.

multaneously. Hadorn and Zürcher (2) also reported that the formation of peroxides and volatile compounds take place simultaneously at 120°C. These authors compared the development of peroxide value and the conductivity of a sunflower oil at 120°C. The good correlation between the results of AOM and the Rancimat method by Läubli and Bruttel (6) indicates the simultaneous formation of peroxides and volatile compounds also.

For chemiluminescence, this result is notable (Table 4) because antioxidants, such as tocopherols, are able to stabilize radicals (25) (either in the sample or produced by the catalyst hemin from hydroperoxides or peroxides) and make them unavailable for the chemiluminescence reaction. These radicals should react with luminol to produce the light. Table 5 demonstrates the strong effect of α- and δ-tocopherol on the chemiluminescence reaction. The results of the chemiluminescence method depend more on the presence of antioxidants in the oil than do the results of the Rancimat (conductivity) method or the iodimetric determination of peroxides (15). Antioxidants prolong the IP of oils but also decrease the chemiluminescence signal (11). This is why a stronger influence of the tocopherol content of the oils on integrals, measured by the chemiluminescence method, would be expected.

Effect of temperature on IP. To show the effect of the operation temperature, the IP were determined for walnut oil with both methods at 100, 110, 120, and 130°C. The experiments carried out at 100°C showed a low slope of the resulting curves, and no definite IP was found. The resulting IP of 110, 120, and 130°C, determined with both methods, were comparable (Table 6, Fig. 2B) ($r = 0.9978$). There were no statistically significant differences between the mean values of the results of these methods ($P < 0.005$). Results in previous Rancimat studies (2,5) showed that an increase of 10°C halved the IP. In this study, the same effect was found for both the Rancimat (conductivity) and the chemiluminescence method (Table 6). The IP determined by chemiluminescence measurements halved (145 → 75 → 38 min) with an increase in temperature of 10°C (110° → 120 → 130°C).

TABLE 6
Induction Periods (IP) of Walnut Oil at Different Temperatures Determined by the Rancimat and Chemiluminescence Methods

Temperature (°C)	Rancimat		Chemiluminescence	
	IP (min)[a]	SD[b]	IP (min)[a]	SD[b]
130	45	6.14	38	8.83
120	84	3.69	75	3.97
110	156	7.31	145	11.52

[a] Average of the IP of quadruplicate runs.
[b] SD (min).

At temperature conditions used in these investigations, saturated fatty acids and their esters remained stable. Only at temperatures exceeding 150°C did they undergo oxidation and decomposition (26). Unsaturated fatty acids are much more susceptible to oxidation, but the principal reaction pathways are basically the same for low and high temperature oxidations (26), even though the oxidation rates increase with temperature because they are complex functions of temperature (10). Therefore, it can be assumed that at temperatures used in these investigations, peroxides, hydroperoxides, and radicals are formed, which are able to react in the chemiluminescence reaction.

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