Validation of the Rancimat Test for the Assessment of the Relative Stability of Fish Oils

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ABSTRACT: The induction periods for the peroxidation of various fish oils at 55–90°C were studied by the Rancimat test. The natural logarithms of the induction periods varied linearly with respect to temperature, with a mean coefficient of -7.5×10^{-2} °C^{-1}, which was significantly different from that reported for vegetable oils. The activation energy for the formation of volatile acids had a mean value of 38.9 kJ/mol and was independent of the fish oil source. Peroxide formation under Rancimat test conditions followed first-order kinetics. The same kinetics were followed under Schaal Oven test conditions (forced-air oven, 60°C). On the basis of the results obtained, the Rancimat test appears to be useful in determining the relative stabilities of fish oils without the change in peroxide decomposition kinetics that may occur at elevated temperatures.
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KEY WORDS: Anchovy oil, fish oil oxidation, hake liver oil, peroxidation kinetics, peroxide decomposition, Rancimat test, Schaal Oven test.

The study of the oxidative stability of fats and oils can be aided by varying parameters, such as temperature, catalyst, or oxygen pressure, to accelerate the peroxidative process (1,2). Among the techniques most commonly used, the Rancimat test has gained acceptance due to its ease of use and reproducibility. In the Rancimat test, an oil sample is heated under atmospheric pressure, and air is allowed to bubble through the oil at a selected temperature (3,4). Under these conditions, the lipoperoxidative process reaches its final steps, and the short-chain volatile acids that are produced are recovered and measured conductimetrically in distilled water (5). The time required to produce a sudden increase of the conductivity due to volatile acids formation determines an induction period (IP), which can be defined as a measure of the stability of a fat or oil.

Depending on the initial concentration of peroxides present in an oil, the lipoperoxidation process can follow two different kinetics. When the initial peroxide content is low, the global rate of peroxide formation has a 0.5 kinetic order with respect to the peroxide concentration. In turn, when the initial peroxide content is high, the global rate of peroxide formation follows first-order kinetics (1).

The Rancimat test has been mainly used with vegetable oils (6–8). However, with this kind of oil sample, high temperatures are required to obtain reasonable IP values. At high temperatures, the mechanisms of peroxidation may be different from those produced at low temperatures; thus, secondary reactions, such as polymer formation, are favored, and the rate of peroxide formation becomes dependent on the oxygen concentration due to its reduced solubility in the oil (1,29). Taking these considerations into account, the conclusions obtained may have a questionable value because they were obtained under conditions that differ from those normally occurring under storage conditions. Thus, the use of high temperatures emerges as a possible limitation of the Rancimat test.

The Rancimat test for fish oils does not require such high temperatures (10,11). The Rancimat model 679 (Metrohm, Switzerland) can be used at temperatures of 50°C, where a change in the mechanisms of lipoperoxidation is not likely to occur.

The objective of the present work was to validate the use of the Rancimat test for the assessment of the relative stability of fish oils. For this purpose the IP dependence on the temperature was established and the kinetics of peroxide and volatile acids formation under Rancimat test conditions was studied with different fish oils.

MATERIALS AND METHODS

Samples. Partially refined Chilean anchovy, Chilean sardine, and Uruguayan hake liver oils were used. Recently prepared anchovy oil (AO) and hake liver oil (HLO) were used with no previous treatment. Sardine oil (SO) was winterized during 4–6 mon at 10°C in a dark glass bottle under a nitrogen atmosphere, then high-vacuum distilled, as previously described (12). AO and SO were a gift from CORPESCA S.A. (Mejillones, Chile), and HLO from ASTRA S.A. (La Paloma, Uruguay). None of the oils studied had antioxidants added.

Rancimat tests. A Rancimat model 679 was used. The IP were determined automatically by the apparatus, and the slopes of the curves beyond the IP were manually determined. The tests were carried out in triplicate, with 3.00 ± 0.05 g of

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oil at selected temperatures (range: 55–90°C) at an air flow of 20 L/h.

The glassware was rigorously cleaned between each run to avoid any contamination that would catalyze the peroxidation. The tubes were cleaned with absolute ethanol, and then with detergent and hot running water. After this they were immersed in an alcoholic solution of NaOH (6%) during the night. Then they were rinsed with running water and double-distilled water. Clean glassware was thoroughly dried in an oven. Oils were kept in dark glass bottles at 4°C under a nitrogen atmosphere between each run.

Sensitivity of the Rancimat test. The principle of determination of the conductivity in the Rancimat test is based on measurement of the resistance (R) of the solution of the recovered volatile acids. Assuming that the solution behaves as a homogeneous conductor, the resistance of the solution could be expressed as:

$$R = \sigma\left(\frac{l}{S}\right) \quad [1]$$

where σ is the resistivity, l is the distance between the electrodes, and S the electrode section. The apparatus electronically calculates the conductance L, defined as the inverse of the resistance:

$$L = \left(\frac{1}{\sigma}\right)\left(\frac{S}{l}\right) \quad [2]$$

The parameter l/σ is defined as the conducitivty, χ, which is reported by the apparatus as a function of the time. The ratio l/S is defined as the cell constant, k. The equation for conductivity is then:

$$\chi = L \cdot k \quad [3]$$

Thus, for reporting the oxidation curve, the apparatus transforms the resistance measurements into conductivity values by using the cell constant values. The IP is determined by performing the second derivative of the conductivity, $\partial^2\chi/\partial t^2$. If the variations are not sensitive enough, the apparatus does not detect the IP. The equipment allows manual changes of the cell constant values, as they should be calibrated after a long period of usage.

Equation 3 shows that duplication of the cell constant value leads to a duplication in the conductivity values. This was advantageously used as a simple way to increase the sensitivity of the test. Table 1 shows that the IP could not be detected by the apparatus by using the real values of the cell constant; but when the cell constant was increased by a factor greater than 1.8, the IP was better defined and could be measured. Note that the IP values were not significantly altered by this procedure. In view of these results, the experiments were carried out with cell constant values which were double those of the real ones.

Kinetic studies. Six samples, weighing 3.00 ± 0.05 g, were placed in the Rancimat at 60, 70, and 80°C. At predetermined times, two tubes were taken out and immediately soaked in a water/ice bath to slow the reaction. For each sample, duplicate determinations of peroxide value (POV) were completed. Data were processed to fit kinetic models of 0.5 and first order.

TABLE 1
Effect of Cell Constant Values (k) on the Induction Period (IP) of Sardine Oil at 50°C Assessed by the Rancimat Test

k_{real} (cm^{-1})	k_{used} (cm^{-1})	Factor (k_{used}/k_{real})	IP (h)
0.83	0.83	1	nd[a]
0.84	0.84	1	nd
0.85	1.00	1.2	nd
0.85	1.50	1.8	11.4
0.82	1.80	2.2	11.2

[a]nd = Not detected by the apparatus.

Other kinetics studies were performed by using the Schaal Oven test. SO samples were placed in 250-mL bottles in an oven at 60°C. Two samples were taken at different times, and the POV was determined in duplicate. Data were processed to fit kinetic models of 0.5 and first order.

Chemical analyses. POV were determined according to AOCS method Cd 8-53 (13). Fatty acid composition was assessed by gas chromatography of their methyl esters, obtained according to AOCS method Ce 1b-89 (13). A Carlo Erba (Milan, Italy) GC-6000 Vega-2 gas chromatograph was used. Fatty acids were analyzed with a wide-bore column, 30-m in length and 0.75-mm i.d., with SP-2330 as stationary phase (Supelco Inc., Bellefonte, PA). The temperature program was 5 min at 150°C, then the temperature was increased at 2°C/min to 190°C and 3°C/min to 211°C, and 10 min at 211°C. Injection and flame-ionization detector temperatures were 250 and 270°C, respectively. Nitrogen was used as the carrier gas. A Spectra-Physics SPA 4270 integrator (Freman, CA) was used to determine area percentages.

Statistical analyses. Variance homogeneity was assessed with the Bartlett test (14). The significance of correlation coefficients for the lineal regression models were tested by the Student's t-test (14). A significance level of 5% was considered significant for all statistical analyses.

RESULTS AND DISCUSSION

The main components of the oils studied are summarized in Table 2. The percentages of saturated fatty acids (SAT) and monounsaturated fatty acids (MUFA) for AO and SO were similar, whereas HLO had a greater relative proportion of MUFA due to relatively great quantities of 20:1 and 22:1. Total polyunsaturated fatty acids (PUFA) for the three oils were similar, although eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are significantly greater for AO and SO ($P < 0.05$) because of the presence of other PUFA in HLO, such as 18:4n-3 and 20:4n-6. From the information stated above, the PUFA/SAT ratio was greatest for HLO, followed by SO and AO.

IP. Table 3 summarizes IP values for the different oils at different temperatures. As expected, the IP values decreased

TABLE 2
Characteristics and Fatty Acid Composition of the Soils Studied

	Anchovy oil	Hake liver oil	Sardine oil
Partially refined	+	+	+
Winterized	–	–	+
Vacuum-distilled	–	–	+
Fatty acids[a]			
14:0	7.2 ± 0.1	6.8 ± 0.4	7.7 ± 0.3
16:0	17.6 ± 0.05	16.7 ± 1.2	17.5 ± 0.5
18:0	4.0 ± 0.5	1.2 ± 0.0	3.3 ± 1.0
SAT	28.8	24.7	28.5
16:1	8.3 ± 0.2	7.8 ± 0.4	8.5 ± 0.2
18:1	13.5 ± 0.3	17.2 ± 0.5	13.1 ± 0.1
20:1	2.2 ± 0.1	4.8 ± 0.1	2.4 ± 0.0
22:1	trace	1.3 ± 0.2	trace
MUFA	24.0	31.1	24.0
18:2n-6	1.2 ± 0.0	2.0 ± 0.2	2.5 ± 0.3
18:4n-3	1.6 ± 0.0	3.5 ± 0.2	1.2 ± 0.0
20:4n-6	1.6 ± 0.1	4.7 ± 1.1	1.0 ± 0.1
20:5n-3	14.9 ± 0.1	7.5 ± 0.4	18.1 ± 1.0
22:5n-3	2.3 ± 0.2	0.9 ± 0.1	2.6 ± 0.2
22:6n-3	12.1 ± 0.5	15.5 ± 1.3	10.9 ± 0.6
PUFA	33.7	34.1	36.3
Total reported	86.5	89.9	88.8
EPA + DHA[b]	27.0	23.0	29.0
PUFA/SAT	1.17	1.38	1.27

[a]Expressed as mass percentage. Mean values ± one standard deviation. SAT, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
[b]EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3).

as the temperature increased. The natural logarithms of the IP varied linearly with temperature ($P < 0.05$) for the three oils studied (Fig. 1). The slopes of the curves in Figure 1 (Table 3), which represent the temperature coefficients for the natural logarithms of the IP, did not differ significantly ($P < 0.05$). This enabled us to establish a common temperature coefficient for the three oils, which, according to our results, had a mean value of -7.5×10^{-2}°C^{-1}. Hasenhuettl and Wan (8) obtained temperature coefficient values for vegetable oils between -2.78×10^{-2} and -3.15×10^{-2}°C^{-1} (mean value: -3.01×10^{-2}°C^{-1}). This could be interpreted as a mean value representative of vegetable oils. Results by Reynhout (7) with soybean oil samples are in agreement with this hypothesis. Although this author did not report the slopes, the values could be easily calculated from the graphs, yielding a value of approximately -3×10^{-2}°C^{-1}, in the range of 80–140°C.

TABLE 3
Induction Period (IP) Values (mean of three analyses ± one standard deviation) for Anchovy Oil, Hake Liver Oil, and Sardine Oil Assessed by the Rancimat Test

Temperature (°C)	IP (h)		
	Anchovy oil	Hake liver oil	Sardine oil
55	21.8 ± 0.8	9.1 ± 0.3	9.7 ± 0.1
60	14.2 ± 0.4	6.3 ± 0.2	6.7 ± 0.1
70	6.5 ± 0.1	2.7 ± 0.1	2.6 ± 0.1
80	3.2 ± 0.0	1.4 ± 0.0	1.5 ± 0.0
90	1.5 ± 0.1	0.7 ± 0.0	0.7 ± 0.0

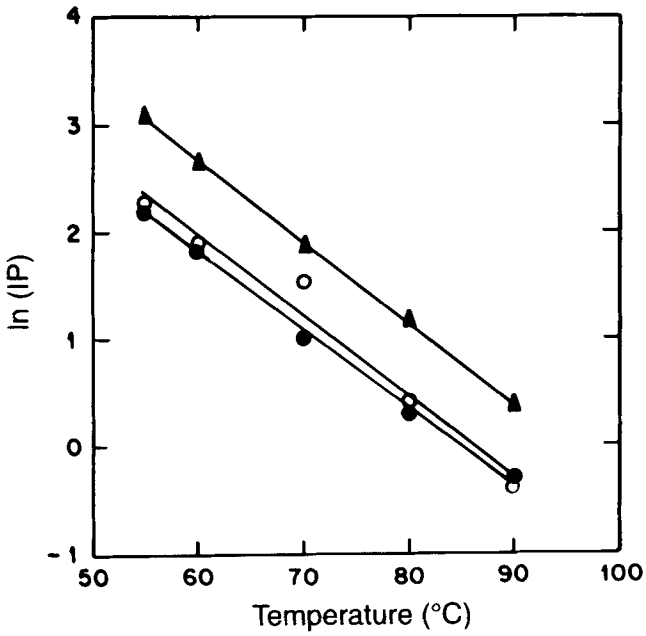

FIG. 1. Linear relationship between the natural logarithm of the induction period (IP) [ln (IP)] assessed by the Rancimat test and the temperature (°C) for anchovy (▲), sardine (○), and hake liver (●) oils.

The intercepts of the curves in Figure 1, obtained for HLO and SO, did not differ significantly; however, the intercept for AO differed from that of HLO and SO (Table 4) ($P < 0.05$). To know which factor was responsible for this difference, a matrix correlation was determined between the intercepts for each curve and the POV, % (EPA + DHA), and PUFA/SAT ratio. Although the number of oils studied was not sufficient to establish the significance of the correlations, the results suggest a possible interpretation. The highest correlation (in absolute value) was with the PUFA/SAT ratio ($r = -0.917$), as compared to % (EPA + DHA) ($r = 0.369$) and POV ($r = -0.442$). It is known that the percentage of SAT in fish oils is usually constant among species and for a single species in different periods of the year (15,16). Generally, variations in the fatty acid composition are mainly due to the MUFA and PUFA. For this reason, the PUFA/SAT ratio (also known as polyene index) is usually taken as a measure of the extent of polyunsaturation of an oil and, obviously, of its tendency to undergo autoxidation.

TABLE 4
Linear Regression Analysis Data for the Relationship Between ln (IP) and Temperature (°C) for Anchovy, Hake Liver, and Sardine Oils[a]

Fish oil	b ± SD	m ± SD (×10^2)	r	P
Anchovy oil	7.3 ± 0.1	−7.6 ± 0.1	0.9995	<0.05
Hake liver oil	6.2 ± 0.1	−7.3 ± 0.1	0.9979	<0.05
Sardine oil	6.4 ± 0.1	−7.5 ± 0.2	0.9967	<0.05

[a]The regression equation is ln (IP) = m · (temperature, °C) + b, where m is the slope and b is the intercept; r represents the correlation coefficient of the regression line and P the probability. Abbreviation as in Table 2.

The intercepts of the curves of Figure 1 represent the natural logarithms of the IP at 0°C. Taking the IP as a stability criterion, our results showed that the stability order for the oils studied here was AO > SO ≈ HLO. As stated previously, the slopes of the three curves in Figure 1 did not differ significantly, so it can be assumed that they are parallel among them. In this way, the stability order mentioned here would be valid for any temperature in the range studied.

Slope of the Rancimat curve beyond the IP. Most of the volatile acid components produced in the Rancimat test are formic acid, with lesser amounts of acetic, propionic, and other acids (5). deMan *et al.* (5) showed that the curve for the production of formic acid is reasonably coincident with the curve of the Rancimat beyond the IP. In this way, the Rancimat slope beyond the IP is proportional to the rate of volatile acid formation. When these slopes were calculated, and their natural logarithms were plotted vs. the inverse of the absolute temperature, a relationship similar to the Arrhenius model was obtained ($P < 0.05$) for the three oils (Fig. 2). The Arrhenius activation energy for the volatile acids production for each oil [calculated as the slope of the plot of ln (slope) vs. 1/T, multiplied by 8.314 J/mol · K] did not differ among them ($P < 0.05$) and yielded a mean value of 38.9 kJ/mol. This implies that the production of volatile acids was not dependent on the fish oil source, so it did not affect the assessment of the relative stability of fish oils.

Kinetics of peroxide decomposition under Rancimat conditions. The Rancimat test for vegetable oils implies a need for high temperature (between 100–140°C), at which the lipid peroxidation mechanisms involved may differ with respect to those operating at room temperature. For fish oils, temperatures under 100°C are adequate for obtaining measurable IP,

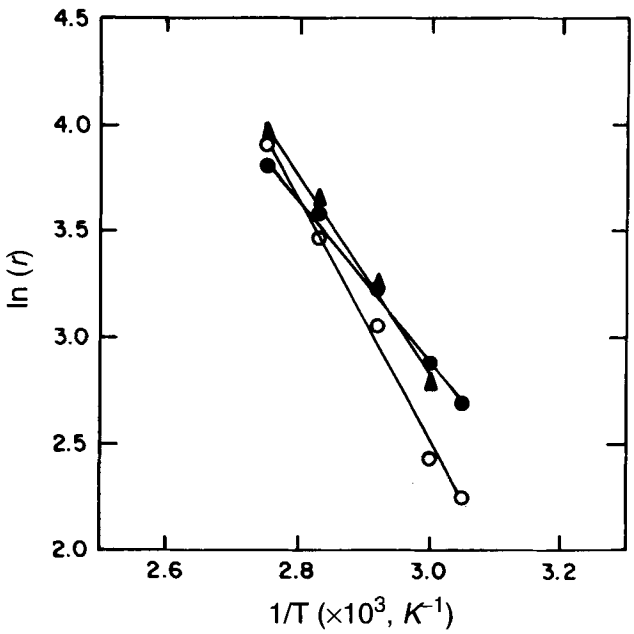

FIG. 2. Arrhenius plot for the rates of volatile acids formation (*r*) for anchovy (▲), sardine (○), and hake liver (●) oils.

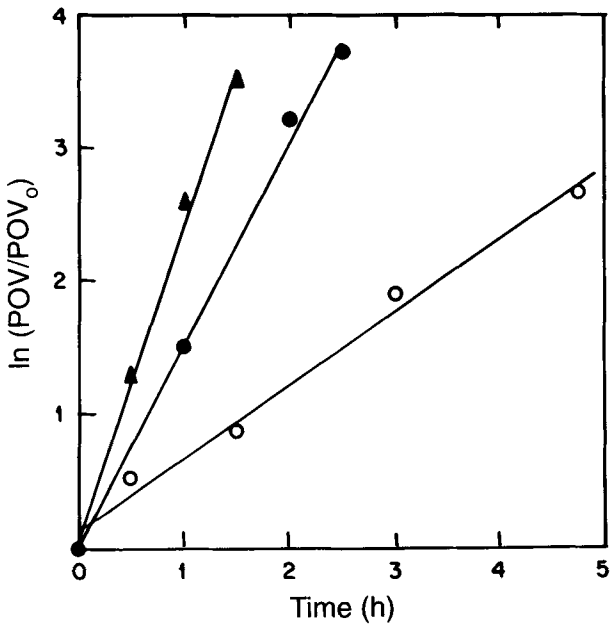

FIG. 3. Kinetics of peroxide decomposition for sardine oil under the conditions of the Rancimat test (see Material and Methods section) at 60°C (○), 70°C (●), and 80°C (▲). POV, peroxide value; POV_0, initial peroxide value.

as shown in this study. It is reasonable to suppose that the mechanisms of lipoperoxidation in the temperature range studied did not change with respect to those operating at lower temperatures. To prove this, the rate of peroxide formation (before the IP) was determined under the conditions of the Rancimat test, as described in the Materials and Methods section. A linear relationship was obtained between ln (POV/POV_0) vs. time at 60, 70, and 80°C (Fig. 3), which implies first-order kinetics. In other studies, SO samples were subjected to the Schaal Oven test (forced-air oven at 60°C). The Schaal Oven test method is considered to correlate well with normal storage conditions (1). Results showed that, under these conditions, the peroxide decomposition followed first-order kinetics, and thus represents those obtained under Rancimat test conditions. Under the experimental conditions employed here, the Rancimat test is a useful tool for assessing the relative stabilities of fish oils.

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REFERENCES

1. Labuza, T.P., Kinetics of Lipid Oxidation in Foods, *CRC Crit. Rev. Food Technol. 2*:355–405 (1971).
2. Frankel, E.N., In Search of Better Methods to Evaluate Natural Antioxidants and Oxidative Stability in Food Lipids, *Trends in Food Sci. & Technol. 4*:220–225 (1993).

3. deMan, J.M., and L. deMan, Automated AOM Test for Fat Stability, *J. Am. Oil Chem. Soc. 61*:534–536 (1984).
4. Laubli, M., and P. Bruttel, Determination of the Oxidative Stability of Fats and Oils: Comparison Between the Active Oxygen Method (AOCS cd 12-57) and the Rancimat Method, *Ibid. 63*:792–795 (1986).
5. deMan, J.M., F. Tie, and L. deMan, Formation of Short Chain Volatile Organic Acids in the Automated AOM Method, *Ibid. 64*:993–996 (1987).
6. Gutiérrez Rosales, F., Determinación de la Estabilidad Oxidativa de Aceites de Oliva Vírgenes: Comparación Entre el Método de Oxígeno Activo (AOM) y el Método Rancimat, *Grasas y Aceites 40*:1–5 (1989).
7. Reynhout, G., The Effect of Temperature on the Induction Time of a Stabilized Oil, *J. Am. Oil Chem. Soc. 68*:983–984 (1991).
8. Hasenhuettl, G.L., and P.J. Wan, Temperature Effect on the Determination of Oxidative Stability with the Metrohm Rancimat, *Ibid. 69*:525–527 (1992).
9. Ke, P.J., and R.G. Ackman, Bunsen Coefficient for Oxygen in Marine Oils at Various Temperatures Determined by and Exponential Dilution Method with Polarographic Oxygen Electrode, *Ibid. 50*:429–435 (1973).
10. Pacheco, M.T.P., Obtencao e Fracionamento do Óleo do Figado do Tiburao Azul *(Prionace glauca)* e Sua Estabilizacao com Antioxidante, M.Sc. Thesis, Facultade de Engenharía de Alimentos, Universidade Estadual de Campinas, 1991.
11. Rodríguez, A., D. Barrera-Arellano, and M.A. Grompone, Estabilidad Oxidativa de Aceite de Hígado de Merluza, *Grasas y Aceites 44*:270–274 (1993).
12. Dinamarca, E., F. Garrido, and A. Valenzuela, Simple High Vacuum Distillation Equipment for Deodorizing Fish Oil for Human Consumption, *Lipids 25*:170–171 (1990).
13. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 4th edn., edited by R.E. Walker, American Oil Chemists' Society, Champaign, 1991.
14. Demczylo, V., J. Geille, and V. Martínez, *Estadística,* Ediciones de la Universidad de la República, 1984, Montevideo.
15. Ackman, R.G., Fish Lipids, Part 1, in *Advances in Fish Science and Technology,* edited by J.J. Connel, Fishing News Book Ltd., Farnhan, Surrey, 1980, pp. 86–103.
16. Ackman, R.G., Fatty Acid Composition of Fish Oils, in *Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oils,* edited by S.M. Barlow and M.E. Stansby, Academic Press, London, 1982, pp. 25–88.

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