Comparison of Rancimat Evaluation Modes to Assess Oxidative Stability of Fish Oils

Eduardo Méndez[a], Julio Sanhueza[b], Hernán Speisky[b], and Alfonso Valenzuela[b,]*

[a]Facultad de Ingeniería, Instituto de Química, Universidad de Uruguay, Montevideo, Uruguay, and [b]Unidad de Bioquímica Farmacológica y Lípidos, INTA, Universidad de Chile, Santiago, Chile

ABSTRACT: Two Rancimat evaluation modes, the induction period (IP), and the time needed to achieve a selected difference in conductivity (tΔK) were compared for assessing relative stability of anchovy, sardine, and hake liver oils. Mean coefficients of variation were 2.5 and 2.4% for IP and tΔK values, respectively, for oils oxidized in the range 55–90°C. Natural logarithms of IP and tΔK values varied linearly with temperature ($P < 0.001$). A linear relationship ($r = 0.999$) was established between the IP and tΔK values ($P < 0.001$). Relative oxidative stability of fish oils was determined with the same degree of confidence by either IP or tΔK values.

JAOCS 74, 331–332 (1997).

KEY WORDS: Conductivity difference, fish oil, induction period, oxidative stability, rancidity, Rancimat.

The Rancimat apparatus is an accelerated method to assess oxidative stability of fats and oils (1). In this test, the oil sample is placed in a vessel, subjected to an above-ambient temperature while oxygen is bubbled through to accelerate the oxidative process into its final steps where short-chain volatile acids are produced. In a separate vessel, the acids formed are immediately dissolved in distilled water, and the conductivity of this solution is monitored at ambient temperature (2,3). The end point of the Rancimat test can be determined by three modes: (i) induction period (IP) to the inflection point in the oxidation curve; (ii) time to delta K (tΔK), which is the time needed to achieve a selected difference in conductivity; or (iii) delta t, when the conductivity is measured at a selected time of the test (1). IP is widely used, although tΔK offers a more rapid acquisition of results. IP values were useful to establish the relative stability of fish oils, and the natural logarithms of IP varied linearly with temperature (4). In this work, relative oxidative stabilities of hake liver oil, whole anchovy oil, and sardine oil were measured by the IP and tΔK evaluation modes.

MATERIALS AND METHODS

Partially refined whole anchovy (AO), whole sardine (SO), and hake liver (HLO) oils with no added antioxidants were used. AO and SO were gifts from Corpesca S.A. (Mejillones, Chile), and HLO from Astra S.A. (La Paloma, Uruguay). Fatty acid compositions were previously reported (4). The Rancimat, model 679 (Metrohm, Herisau, Switzerland) was used with two evaluation modes, i.e., IP and tΔK modes, which were conducted simultaneously. The latter was set at 50 $\mu S \cdot cm^{-1}$, representing a signal/baseline ratio of 25. Tests were carried out in triplicate on 3.0 ± 0.05 g of each oil at selected temperatures (range of 55–90°C) and with an airflow of 20 L/h.

Correlations were calculated by least-squares regression analysis. The linearity of the fitted curves was assessed by the F-test, and the validity of the coefficients was determined by the T-test (5).

RESULTS AND DISCUSSION

The mean coefficients of variation were 2.5% (range 1.1–3.6%) for the IP values and 2.4% (range 1.8–3.9) for the tΔK values (Table 1). Parameters resulting from the linear regression analysis of ln (IP) and ln (tΔK) vs. temperature showed that natural logarithms of both evaluation modes varied linearly with temperature ($P < 0.001$), as revealed by the high F values (Table 2). At 60, 70, and 80°C, the temperature coefficients were also statistically significant ($P < 0.001$), and therefore useful to estimate IP and tΔK values, as shown by the T-test values. The intercepts of both regression lines represent ln (IP) and ln (tΔK) at 0°C, and the slopes of the regression lines for each evaluation mode are parallel ($P < 0.05$), independently of the fish oil studied. Thus, under these experimental conditions, the intercepts could be confidently taken as stability criteria. Both evaluation modes led to the same stability order: AO > SO ≈ HLO in the temperature range studied.

The relationship between IP and tΔK values was assessed by applying linear regression analysis to the data (Table 3). F-test and T-test values showed this relationship to be signifi-

---

*To whom correspondence should be addressed at Unidad de Bioquímica Farmacológica y Lípidos, INTA, Universidad de Chile, Casilla 138-11, Santiago, Chile.

TABLE 1
Mean Values ($n = 3$) and Standard Deviations (in parentheses) for the Induction Period (IP) and the Time to Delta K (tΔK) Evaluation Modes for Assessment of Relative Oxidative Stability of Fish Oils

	Anchovy oil		Hake liver oil		Sardine oil	
Temperature (ºC)	IP (h)	tΔK (h)	IP (h)	tΔK (h)	IP (h)	tΔK (h)
55	21.7 (0.8)	24.3 (1.0)	9.1 (0.3)	11.9 (0.4)	9.7 (0.1)	14.3 (0.2)
60	14.2 (0.4)	16.8 (0.5)	6.3 (0.2)	8.9 (0.4)	6.7 (0.1)	10.1 (0.1)
70	6.5 (0.1)	7.4 (0.1)	2.7 (0.1)	4.5 (0.1)	2.6 (0.1)	4.6 (0.2)
80	3.2 (0.0)	3.9 (0.1)	1.4 (0.0)	2.3 (0.0)	1.5 (0.0)	2.6 (0.2)
90	1.5 (0.1)	2.1 (0.0)	0.7 (0.0)	1.3 (0.0)	0.7 (0.0)	1.3 (0.0)

TABLE 2
Parameters of Linear Regression Analysis for the Dependence of the Natural Logarithms of the Induction Period (IP) and the Time to Delta K (tΔK) on Temperature (range 55–90ºC)[a]

Fish oil	Evaluation mode	Slope (°C-1)	Intercept (h)	Correlation coefficient	F-test	T-test
Anchovy	IP	–0.076 (0.001)[a]	7.3	0.9995	5487*	–74.1*
	tΔK	–0.070 (0.002)[b]	7.0	0.9983	898*	–30.0*
Hake liver	IP	–0.073 (0.001)[a]	6.2	0.9979	872*	–29.5*
	tΔK	–0.064 (0.002)[b]	6.0	0.9992	1908*	–43.7*
Sardine	IP	–0.075 (0.002)[a]	6.4	0.9967	509*	–22.6*
	tΔK	–0.068 (0.002)[b]	6.3	0.9988	1200*	–34.6*

[a] Results are the average of three independent runs, and standard deviations are in parentheses. Slopes with the same superscript (a,b) did not differ significantly ($P > 0.05$); *significant at $P < 0.001$.

TABLE 3
Statistical Parameters of the Relationship Between the Induction Period and the Time to Delta K Evaluation Modes in the Analysis of the Relative Stability of Fish Oils in the Range 55–90ºC[a]

Parameter	Anchovy oil	Hake liver oil	Sardine oil	Pooled data
Slope	0.897	0.789	0.704	0.872
Intercept	–0.36	–0.52	–0.39	–0.86
Correlation coefficient	0.9994	0.9979	0.9991	0.9899
F-test	2347*	704*	1721*	631*
T-test	48.5*	26.5*	41.5*	25.1*

[a] Data with an asterisk (*) are significant at $P < 0.001$.

cant to 0.1% confidence. Pooled fish oil data also showed the same pattern of a linear relationship.

We have recently reported (4) that the rate of volatile acid formation and activation energy was similar among the three studied oils (mean calculated value was 38.9 kJ/mol). Because the rates of increment of the conductivity appear not to be different among the three fish oils, the tΔK values could be used with the same confidence as the IP values to assess relative stability of fish oils. In addition, the time required to obtain the tΔK values was *ca.* 25% less than that needed to obtain IP values. That difference might be explained on the basis of the differences in the conductivity values needed to detect the end points with the two evaluation modes. While a conductivity of 250 $\mu S \cdot cm^{-1}$ was needed with the IP mode, a conductivity of only 50 $\mu S \cdot cm^{-1}$ was required when using the tΔK mode.

ACKNOWLEDGMENT

These studies were partially funded by Fondecyt-Chile, Grants # 1950258 and # 1930808.

REFERENCES

1. Cabré Cabré, P., and A. Massó Maresch, *Determinación de la estabilidad a la oxidación de aceites y grasas por el método Rancimat*, Masso Analítica, Barcelona, España, 1982.
2. deMan, J.M., and L. deMan, Automated AOM Test for Fat Stability, *J. Am. Oil Chem. Soc. 61*:534–536 (1984).
3. 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).
4. Méndez, E., J. Sanhueza, H. Speisky, and A. Valenzuela, Validation of the Rancimat Test for the Assessment of the Relative Stability of Fish Oils, *Ibid. 73*:1033–1037 (1996).
5. Demczylo, V., J. Geille, and J. Martinez, *Estadística*, Ediciones de la Universidad de la República, Montevideo, Uruguay, 1984.

[Received April 30, 1996; accepted December 11, 1996]