Determination of Wax Concentration in Sunflower Seed Oil

S. Martini* and M.C. A n

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) (UNLP-CONICET),
La Plata 1900, Provincia de Buenos Aires, Argentina

ABSTRACT: Isothermal crystallization of waxes was studied by using an optical setup. The induction time of crystallization was assessed as a function of wax concentration. The relationship was found to be a decreasing exponential curve. The wax content of some of the solutions prepared in the laboratory was determined by calculating the crystallization induction time. The values obtained were compared to those from different methods (cold test, microscopic, and turbidimetric methods). The results obtained with the optical setup method are similar to those obtained with other methods for concentrations greater than 100 ppm. An analysis of variance test was used to verify the authenticity of the values obtained with the optical method. Results showed that the method used to determine wax concentration, the concentration of the sample, and the relationship between both parameters do not affect significantly the values of percentage relative errors ($P < 0.05$) obtained for concentrations greater than 100 ppm. Values obtained for wax content within the range 0–100 ppm could not be compared since the microscopic and turbidimetric methods are not sensitive enough, unlike the optical setup, to detect wax amounts in such low concentration.

Paper no. J9397 in *JAOCS 77,* 1087–1092 (October 2000).

KEY WORDS: Cold test, isothermal crystallization, laser, microscopy, optical setup, sunflower oil, turbidimetry, waxes.

The removal of impurities, such as free fatty acids, phospholipids, moisture, and pigments, normally present in crude sunflower oils must include the elimination of waxes that are responsible for the appearance of turbidity in the refined oil, especially when the oil is cooled to temperatures below 20°C (1). The waxes of sunflower oil are esters of long-chain saturated fatty acids (C_{20}–C_{22}) with alcohols of C_{24}–C_{28}. The melting point of sunflower waxes is about 70–80°C. Waxes are not polar at ≥40°C and are lipophilic, but at temperatures under 40°C they acquire a crystalline state with some slight hydrophilic characteristics (2). Waxes are found mainly in the seed hull (1.5–3.0%) and are incorporated into the oil during the pressing and extraction procedures. Values reported in the literature show that wax content in sunflower seed oil is 0.02–0.35% (3–5). Some authors have reported values as high as 1% (6–8). Owing to both a high melting point and a low solubility, the presence of even small quantities of these components in refined oils can cause the presence of a crystalline precipitate that affects the brightness and transparency of the oil. For this reason, waxes must be eliminated, especially if the oil is packed, as normal, in transparent bottles (1). Consequently, good separation of waxes has to be ensured through the formation of crystals that can be filtered or centrifuged easily with minimal retention so as to maintain high yields of refined oil.

Turkulov *et al.* (9) have demonstrated that oils containing 8–70 mg of waxes in 1 kg of oils (8–70 ppm) pass the cold test but develop turbidity if they remain at such low temperatures for 48 h. Several authors have developed different methods to determine wax content in oils. Brimberg and Wretensjo (10) and Morrison and Robertson (3) have developed turbidimetric methods that are less time-consuming than the cold test. Rivarola *et al.* (11) have developed a microscopic method based on the oil solubility of waxes.

The aim of this study was to develop a method to determine wax concentration in refined oil (wax content under 50 ppm). Sunflower oil was used because of its importance in Argentina. The method developed was compared with methods already available (cold test, microscopic, and turbidimetric methods).

MATERIALS AND METHODS

Starting oils. Wax-free oil was obtained from commercial oil (Molinos Río de la Plata S.A., Buenos Aires, Argentina) after centrifugation (60 min, 0°C, 3000 × *g*). Waxes that crystallize at 0°C or higher were eliminated by this procedure. Purified waxes were obtained by filtering crude sunflower seed oil (obtained from tank settlings provided by Molinos Río de La Plata) through a Buchner funnel. The solid residue was washed several times with petroleum ether at 0°C. The waxes obtained were purified by means of successive hot extractions with hexane, followed by centrifugation (30 min, 3000 × *g*, 0°C). Different samples of crude and refined oils were provided by Molinos Río de la Plata S.A., and Guipeba-Ceval S.A. (Rosario, Argentina).

Thermal treatments. Purified waxes were weighed and dissolved in wax-free oil in order to obtain solutions with a wax

---

*To whom correspondence should be addressed at CIDCA, Casilla de Correo 553, (1900) La Plata, Provincia de Buenos Aires, Argentina.
E-mail: mcanon@isis.unlp.edu.ar

Copyright © 2000 by AOCS Press

content between 0 and 1000 ppm. The samples were heated for 90 min at 120°C to ensure complete melting of the waxes. They were introduced in a thermostatic cell at the desired crystallization temperature (12, 22, 30, 35°C), and the induction time was calculated. The induction time was defined as the interval between the time at which crystallization temperature was reached, and the start of actual crystallization (first deviation from the laser baseline signal). (i) In using the turbidimetric method, the samples were heated at 120°C for 30 min to dissolve the waxes. After this period of time, the hot oil was placed into the turbidimeter cell and cooled in an ice-water bath until it reached 21°C. At that time, turbidimetric determinations were made. (ii) For the microscopic method, samples were cooled at 0°C for 48 h to ensure complete crystallization of waxes, after which 70 μL of the sample (previously homogenized) was placed between a slide and cover-slide with the thermocouple to observe the melting procedure.

Isothermal crystallization. The basis of the test was to induce isothermal crystallization of the waxes present in sunflower seed oil. The crystallization process was monitored by using an optical setup (12). Equipment used is a modification of that used by Chong and Sato as previously described (13). A helium–neon laser was used as the light source. The sample was contained in a water-jacketed glass cell. A polarizer was put between the laser and the cell. The temperature of the glass cell was controlled by means of water circulated from a thermostatic water bath. The light transmitted by the crystals passed through the second analyzer placed at the Cross-Nicols position with regard to the first one. This enables the photodiode to detect the occurrence of optically anisotropic wax crystals (13).

Turbidimetric method. Turbidity was measured in nephelometric turbidimetric units (NTU) using a La Motte Model 2008 Turbidimeter (Chestertown, MD). The instrument was calibrated by means of a suspension of formazin as standard solution. NTU values were converted into concentration units with a calibration curve obtained with solutions of different wax contents prepared in the laboratory (Fig. 1).

Microscopic method. A Leitz microscope model Ortholux II (Ernest Leitz Co., Wetzlas, Germany) with a controlled temperature plate was used to follow the solubility of waxes as the temperature increased. The temperature was controlled through a programmable cryostat (Lauda UK 30, Werklauda, Königshofen, Germany). Ethylene glycol in water (3:1) was used as the refrigerant fluid. When the plate reached 5°C, the sample and a 0.05 mm diameter copper-constantan thermocouple connected to a two-channel Gilson potentiometer (Gilson France S.A., Villiers le Bel, France) were placed between a slide and the cover-slide. The refrigerant fluid flow was halted and the temperature of the plate increased at a constant rate by means of a rheostat. The temperature at which the wax–oil equilibrium is reached was determined when the last crystal of wax disappeared. With this value of temperature, wax concentration can be determined by means of an equilibrium curve developed in our laboratory (11,14).

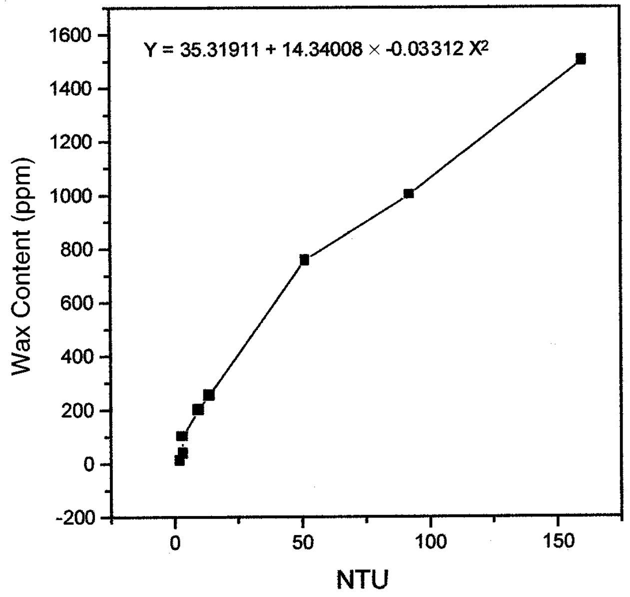

FIG. 1. Calibration curve for the turbidimetric method. NTU, nephelometric turbidimetric units.

Cold test. The cold test was carried out on all samples studied. Each sample was heated at 120°C to ensure complete melting of waxes. They were stored at 0°C for 24 h and the turbidity measures were made.

Statistical study. Solutions of 0, 25, 50, 100, 250, and 750 ppm of waxes in oil were prepared as described before. Duplicates were prepared for each concentration. The wax concentration was determined using the three methods described above and the results were subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The aim of the present study was to develop a method able to determine wax concentration in sunflower seed oil. The basis of the method is the induction of isothermal crystallization of the waxes present in the oil at a given temperature (crystallization temperature). The optical setup used detects the presence of anisotropic wax crystals. The crystallization temperature was controlled by means of water flowing from a thermostatted water bath into a glass cell where the hot sample was placed. The temperatures used in these experiments were chosen on the basis of the solubility curve of waxes in oil developed by Rivarola *et al.* (11). These experimental values were fitted by means of the following equation: $T_e = T_R + a \cdot (y - y_R)^b$, where T_e = wax–oil equilibrium temperature (°C); T_R = reference temperature, 21°C; y_R = reference concentration, 0.005%; and a = 42.49 and b = 0.1761 with r = 0.991. The sample is introduced into the thermostatic cell at the crystallization temperature desired; the photosensor starts recording and the temperature is lowered until it reaches the desired crystallization temperature. During this period the laser signal is constant (laser baseline signal), and it remains unchanged until wax crystals appear; at this time the laser signal increases. The photosensor output is recorded together with the cell temperature (copper-constantan thermocouple).

Figure 2 is a typical plot of the CdS photodiode response and the thermocouple recording of the crystallization of waxes. The induction times of crystallization were calculated by means of this plot as described in the Materials and Methods section. The laser signal depends on both wax concentration and crystallization temperature. The first temperature selected was 12°C, and the induction times for solutions of low wax content (11, 13, 16, 21, 31, 40 ppm) were calculated as the mean of three measurements. When a sample containing 50 ppm wax was assayed at 12°C, the induction time obtained was negative. This means that crystallization had occurred before the desired crystallization temperature was reached. Therefore, the crystallization temperature for this solution should be higher than 12°C. According to the solubility curve of waxes in sunflower seed oil (11), the temperature selected for solutions of 50 ppm of waxes or more was 22°C. Isothermal crystallization of solutions between 50 and 150 ppm (52, 62, 85, 102, 120, 151 ppm) was induced, and induction times were calculated.

To be able to apply isothermal crystallization not only to refined oils but also to semirefined and crude oils, a wider range of concentrations was analyzed. Samples of 200–500 ppm (201, 252, 302, 400, 500 ppm) were measured at 30°C. To measure samples of higher wax content, the temperature used was 35°C, and the samples contained 500, 598, 757, and 1000 ppm. The last two temperatures (30, 35°C) were chosen in the same way as 12 and 22°C.

After all the temperatures were assayed, the induction times determined were plotted vs. wax content for each temperature (Fig. 3). A regression was made with the SYSTAT (Wilkinson, L., SYSTAT, The System for Statistics, SYSTAT Inc., Evanston, IL, 1990) program to the data points obtained. The best correlation found in all cases was an exponential curve:

$$y = A_1 e^{-(x-x_0)/t_1} + A_2 e^{-(x-x_0)/t_2} \qquad [1]$$

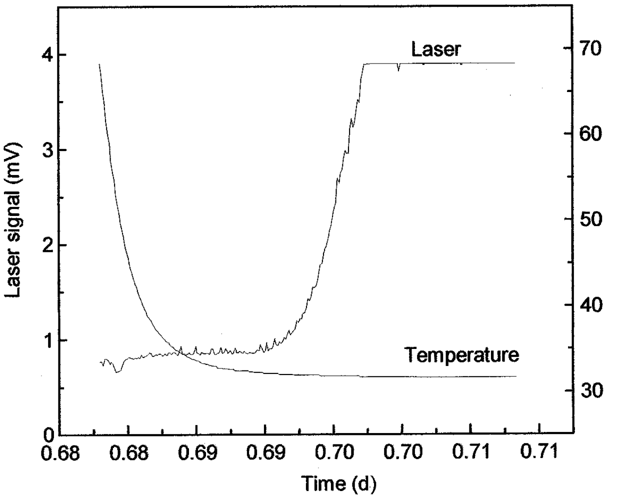

FIG. 2. Crystallization of waxes from purified sunflower oil. Thermocouple and photodiode record as a function of time.

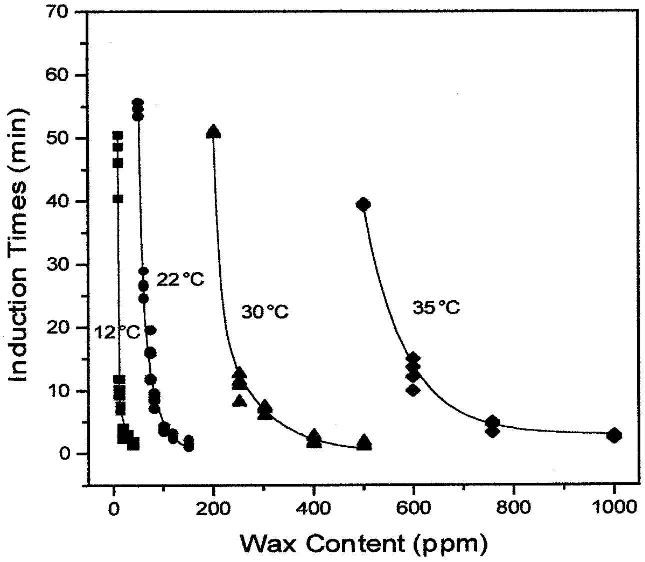

FIG. 3. Induction times as a function of wax content. Induction times were measured at 12°C for solutions with wax content between 0–50 ppm (■); 22°C, 50–150 ppm (●); 30°C, 200–500 ppm (▲); 35°C, 500–1000 ppm (◆).

The values of the constants (A_1, x_0, t_1, A_2, t_2) vary for each temperature (Table 1). By means of these correlation values the wax content of an unknown sample can be determined by calculating its induction time at the corresponding crystallization temperature. In the plots of induction times vs. wax content (Fig. 3), the error at 12°C is larger than at other temperatures. The error in each plot can be analyzed by means of the correlation coefficient of the graphic of ln t as a function of wax content (Fig. 4) when a linear regression is made (Table 2). This is explained by the fact that crystallization temperature varies strongly with wax content. In the solubility curve developed by Rivarola *et al.* (11), solubility increases at a high rate for low concentrations (0–100 ppm), whereas the slope becomes almost nil for higher concentrations.

The concentration of some solutions of waxes in oil prepared in the laboratory were determined with the optical setup method. The concentrations assayed were: 117, 322, and 400 ppm (dil. 1:10). To this end, solutions of 125 and 1500 ppm and some dilutions (1:5, 1:10, and 1:2, 1:5, 1:10, 1:100, respectively) were prepared. The induction times obtained and

TABLE 1
Correlation Values Obtained for the Plot of Induction Time vs. Wax Content

Temperature (°C)	A_1	x_0 (ppm)	t_1 (ppm)	A_2	t_2 (ppm)
12	27.79	11.18	0.66	9.54	12.82
22	32.94	51.59	0.47	41.69	22.55
30	17.67	200.97	106.03	33.51	4.44
35	5.40	505.95	692.73	31.14	67.73

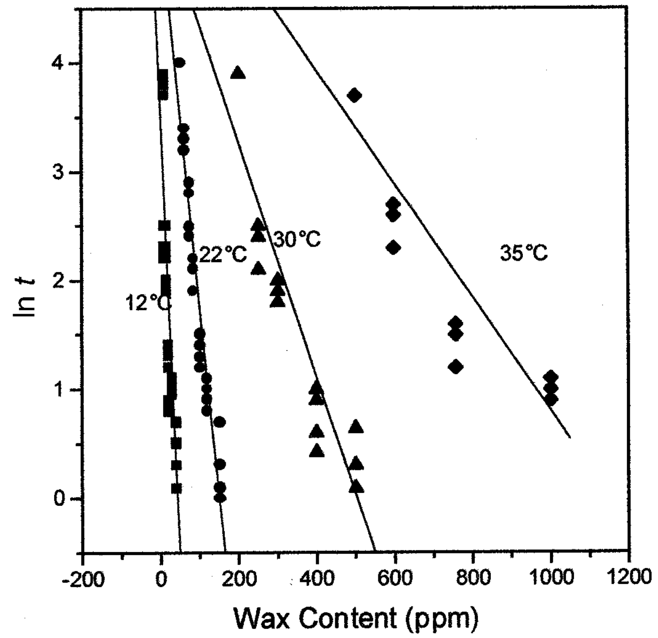

FIG. 4. Induction times of crystallization (ln *t*) as a function of wax content. The logarithm of induction times was measured at 12°C for solutions with wax content of 0–50 ppm (■); 22°C, 50–150 ppm (●); 30°C, 200–500 ppm (▲); 35°C, 500–1000 ppm (◆).

the concentrations calculated are listed in Table 3. To verify whether the optical setup could be compared with the methods developed by other authors, the concentration of solutions of 125 and 1500 ppm and their dilutions were determined by the microscopic and turbidimetric methods. These values were compared with those of the cold-test determinations (Table 4). The three methods lead to similar values for solutions laboratory-prepared of >100 ppm of wax content. The values obtained for solutions below 100 ppm with the turbidimetric method are higher than the ones obtained with the optical setup owing to the low sensitivity of the first one for solutions of 50–100 ppm. When solutions are diluted (<100 ppm), wax crystals cannot be seen with the microscopic method, and no concentration can be determined.

An ANOVA test was performed to prove whether the three methods could really be compared. Duplicate solutions of 0, 25, 50, 100, 250, and 750 ppm of waxes in sunflower seed oil were prepared and their concentrations were determined using the three methods. The values obtained (Table 5) are similar to those of Table 4. The percentage relative error (RE, defined as the difference between the values obtained with each method and the concentration of the solution, expressed as a percentage) was calculated for each solution of 100, 250, and 750 ppm (Table 6). An ANOVA was made of RE using the SYSTAT program and the following factors: the method used to determine wax concentration; the wax content of the sample; and the interaction between the two. The results show that the factors analyzed do not affect significantly the values of RE ($P < 0.05$) obtained between the methods. That is, for concentrations over 100 ppm the three methods are comparable. It was not necessary to apply this test to solutions of low concentration (<100 ppm) because the values obtained were widely different (Table 5).

TABLE 2
Linear Regression Parameters Obtained for the Plot of ln *t* vs. Wax Content[a]

Temperature (°C)	Parameters ($y = A + Bx$)		
	A	*B*	*R*
12	3.70	−0.085	−0.82276
22	5.42	−0.036	−0.97098
30	5.47	−0.011	−0.91173
35	6.04	−0.005	−0.93445

[a] See plot in Figure 4; *t*, induction time.

TABLE 3
Induction Times of Solutions Prepared in the Laboratory[a]

Solutions (ppm)	T_c (°C)	τ_{average} (min)	σ_{n-1}	C_{average} (ppm)	σ_{n-1} (*C*)	C_f (ppm)
117	22	4.13	0.56	104	3	104
322	30	6.27	0.55	311	9	311
$400_{\text{dil 1:10}}$	12	0.775	0.03	43.4	0.6	434
1500	35	—	—	—	—	>1,000
$1500_{\text{dil 1:2}}$	35	4.6	0.2	749	9	1,498
$1500_{\text{dil 1:5}}$	30	8.5	0.1	300.1	32.2	1,500
$1500_{\text{dil 1:10}}$	22	0.99	0.7	139	18	1,390
$1500_{\text{dil 1:100}}$	12	15.3	2.7	12.2	0.3	1,220
125	22	1.3	0.4	130	7	150
$125_{\text{dil 1:10}}$	12	10.3	0.9	12.9	0.2	129
$125_{\text{dil 1:5}}$	12	3.485	0.01	24.10	0.03	120

[a] T_c, crystallization temperature selected; τ_{average}, induction time calculated (mean of three determinations); σ_{n-1}, standard deviation of the induction time obtained; C_{average}, wax concentration calculated with the induction times; σ_{n-1} (*C*), standard deviation of the wax concentration obtained; C_f, final wax concentration of the sample (C_{average} × dilution factor).

The wax content of some samples (refined and crude oils) provided by Molinos Río de La Plata S.A., Argentina and Guipeva-Ceval S.A., Argentina was determined using the three methods listed above (optical setup, turbidimetric, and microscopic methods). Table 7 shows the induction times of the samples (refined, semirefined, and crude oils) and the concentration obtained with the optical setup method. Since some crude oils caused a decrease in the laser signal when crystallization was reached, it was necessary to make a dilution with wax-free oil so that the induction time could be calculated. The impurities present in the oil could be responsible for the different behavior of crude oils from solutions prepared in the laboratory and refined/semirefined oils, since they cocrystallize with waxes when crystallization temperature is reached, thus causing opalescence of the oil. This fact interferes with the photodiode detection, causing a decrease in the laser signal. Table 8 lists the wax contents of the samples obtained using the three methods studied. Wax concentration of crude oils determined with the microscopic method was performed with a 1:10 dilution with wax-free oil, since values obtained with the original samples were too high. This fact can be explained by the presence of impurities that affect the solubility

TABLE 4
Comparison of Different Methods to Determine Wax Concentration in Solutions Prepared in the Laboratory[a]

Sample (ppm)	Microscopy (ppm) $C_{av} \pm \sigma_{n-1}$	Microscopy (ppm) C_f	Optical setup (ppm) $C_{av} \pm \sigma_{n-1}$	Optical setup (ppm) C_f	Turbidimetry (ppm) $C_{av} \pm \sigma_{n-1}$	Turbidimetry (ppm) C_f	Cold test
1500	1120 ± 134	1120	>1000	>1000	1093 ± 5	1093	+++
$1500_{dil\ 1:2}$	674 ± 32	1384	749 ± 9	1498	793 ± 39	1586	++
$1500_{dil\ 1:5}$	319 ± 24	1595	300 ± 32	1500	201 ± 14	1005	+
$1500_{dil\ 1:10}$	159 ± 24	1590	139 ± 18	1390	120 ± 3	1200	—*
$1500_{dil\ 1:100}$	No crystals found		12.2 ± 0.3	1220	94.0 ± 7.0	9400	—
125	124 ± 18	124	130 ± 7	130	138 ± 4	138	+
$125_{dil\ 1:5}$	No crystals found		24.1 ± 0.03	120.5	78.0 ± 3.8	390	—
$125_{dil\ 1:10}$	No crystals found		12.9 ± 0.2	129	81.0 ± 2.0	810	—

[a] $C_{average}$, average wax concentration calculated with the induction times; σ_{n-1}, standard deviation of the wax concentration obtained; C_f, final wax concentration of the sample ($C_{average}$ × dilution factor); —, clear oil; —*, slight turbidity; +, turbidity; ++, visible turbidity; +++, more pronounced turbidity.

TABLE 5
Wax Content (ppm) Values Obtained from Solutions Prepared in the Laboratory

Sample concentration	Microscopy	Optical setup	Turbidimetry
0	No crystals found	<10	68.1
0	No crystals found	<10	78.0
28.2	No crystals found	20.5	68.1
27.0	No crystals found	24.4	63.9
53.0	68.3	51.2	76.6
55.2	60.3	54.1	72.4
100	93.8	102	93.6
101	90.3	108	90.7
252	291	235	249
251	238	237	236
752	706	813	689
750	812	764	736

of waxes in the oil (a fundamental principle of the microscopic method). When wax concentration was determined on refined samples (<100 ppm), crystals were too small to be detected by the optical microscope and the wax concentration could not be determined. In some cases, for wax contents under 100 ppm microscopic and turbidimetric methods gave greater values than the optical setup method, owing to the fact that the microscopic and turbidimetric methods are unable to detect wax content differences between 50 and 100 ppm. These two methods are not sensitive enough to determine wax content for very dilute samples (0–100 ppm) since they can only detect crystals ≥2 μm in diameter (crystallization growing step). The optical setup method can detect this crystallization nucleation step, which enables the detection of lower wax contents (<50 ppm). Although the optical setup method was

TABLE 6
Comparison of Different Methods by Analysis of Variance

Method[a]	Real concentration (ppm)	Measured concentration (ppm)	RE (%)
A	100	93.8	−6.2
B	100	102	2.0
C	100	93.6	−6.4
A	101	90.3	−10.6
B	101	108	6.9
C	101	90.7	−10.2
A	252	291	15.5
B	252	235	−6.7
C	252	249	−1.2
A	251	238	−5.2
B	251	237	−5.6
C	251	236	−6.0
A	752	706	−6.1
B	752	813	8.1
C	752	689	−8.4
A	750	812	8.2
B	750	764	1.9
C	750	736	−1.9

[a] A, microscopic method; B, optical setup method; C, turbidimetric method; RE, difference between the values obtained with each method and the concentration of the solution, expressed as a percentage.

TABLE 7
Determination of Induction Times of Samples

Samples	T_c (°C)	$\tau_{average}$ (min)	σ_{n-1}	$C_{average}$ (ppm)	σ_{n-1} (C)	C_f (ppm)
1[a]	22	12.5	2.6	79.1	4.8	791
2[a]	22	14.9	2.7	75.0	4.1	750
3[a]	22	6.1	0.7	95.0	2.7	950
4[a]	22	12.0	0.2	12.55	0.07	125
5[a]	22	11.5	0.6	80.7	1.2	807
6[a]	22	23.8	1.9	65.9	0.5	659
7[a]	22	16.1	2.3	73.2	3.4	732
8[a]	12	1.265	0.01	37.1	0.1	371
9[a]	22	17.5	1.1	71.2	1.5	712
10	12	8.4	0.5	13.7	0.3	137
11	12	12.9	1.4	12.4	0.2	12.4
12	12	9.2	0.6	13.3	0.2	13.3
13	12	∞[b]	—	—	—	<10
14	12	13.1	—	13.5	—	13.5
15	12	∞[b]	—	—	—	<10
16[a]	22	7.0	0.7	91.9	2.2	919
17[a]	12	9.4	0.7	13.3	0.3	133
18[a]	22	23.3	7.7	65.5	7.4	655
19	22	8.5	1.9	86.8	4.9	86.8

[a] Values measured with a 1:10 dilution of the crude oil.
[b] No crystallization was reached in a period of 2 h of measurement. For abbreviations see Table 3.

TABLE 8
Comparison of Different Methods for Commercial Samples

Sample number	Concentration (ppm)			Cold test
	Microscopy	Optical setup	Turbidimetry	
1	714 ± 62[a]	791 ± 48	757 ± 45	+++
2	740 ± 63[a]	750 ± 41	623 ± 56	+++
3	1128 ± 29[a]	950 ± 27	1272 ± 110	+++
4	880 ± 130	764 ± 9	704 ± 73	++
5	826 ± 17[a]	807 ± 12	780[b]	++
6	653 ± 31[a]	659 ± 5	676 ± 43	++
7	777 ± 14[a]	732 ± 34	988 ± 70	+++
8	No crystals found[a]	371 ± 1	411 ± 28	+
9	656[b]	712 ± 15	610 ± 93	++
10	168 ± 35	137 ± 3	137 ± 22	+
11	70.2 ± 6	12.4 ± 0.4	64.8 ± 1.6	—
12	64.3 ± 7	13.3 ± 0.2	62.0 ± 1.7	—
13	52.0 ± 1.4	< 10 ppm	68.6 ± 3.6	—
14	No crystals found	13.5[b]	93.1 ± 45	—
15	No crystals found	< 10 ppm	—	—
16	997 ± 89[a]	919 ± 22	946 ± 12	+++
17	186 ± 19	133 ± 3	197 ± 2	+
18	637 ± 22[a]	668 ± 41	769 ± 8	+++
19	162 ± 9	86.8 ± 4.9	208 ± 17	+

[a] Values measured with a 1:10 dilution of the crude oil.

[b] Only one determination was made for these samples. —, clear oil; +, turbidity; ++, visible turbidity; +++, more pronounced turbidity.

developed to determine low wax concentrations, it could not detect wax contents below 10 ppm. When a solution of wax in oil below 10 ppm is assayed at 12°C, wax does not crystallize (the laser signal remains constant) and no induction time can be calculated. In this case, another crystallization temperature should be assayed (T_c < 12°C). The method studied in the present work is not sensitive enough when the induction times are too low (<1 min). This should occur for concentrations in the extremes of the interval of each crystallization temperature (40–50, 150, 500, and 1000 ppm, approximately).

ACKNOWLEDGMENTS

This work was supported by funds from SECYT (Secretaría de Ciencia y Tecnología) (Prog. BID 802 OC-AR/ PID 0357), Argentina. Thanks are extended to the National Research Council of Argentina for the Ph.D. scholarship granted to Silvana Martini.

REFERENCES

1. Bloch, S., Distintas Alternativas para la Refinación y Descerado del Aceite de Girasol, *Aceites Grasas 30*:53–66 (1998).
2. Kehse, W., Winterization of Sunflower Oil, *Rev. Fr. Corps Gras 27*:567–570 (1980).
3. Morrison, W.H., III, and J. Robertson, Solvent Winterization of Sunflower-seed Oil, *J. Am. Oil Chem. Soc. 52*:148–150 (1975).
4. Morrison, W.H., III, Rapid Determination of Wax in Sunflower Seed Oil, *Ibid. 59*:284–285 (1982).
5. Ostric-Matijasecic, B., and J. Turkulov, Determination of Waxes in Sunflower Grains, Shells and Oil. Modified Method of Extraction, *Rev. Fr. Corps Gras 20*:5–10 (1973).
6. Ruckenstein, C., Modern Processing of Crude Sunflower Oil, *Ibid. 28*:161–164 (1981).
7. Klein, K., and L.S. Crauer, Further Developments in Crude Oil Processing, *J. Am. Oil Chem. Soc. 51*:382A–385A (1974).
8. Leibovitz, Z., and C. Ruckestein, New Processes in Degumming, Bleaching, Deacidification-Deodorization and Winterizing of Edible Oils, *Rev. Fr. Corps Gras 28*:303–308 (1981).
9. Turkulov, J., E. Dimic, D. Karlovic, and V. Vuska, The Effect of Temperature and Wax Content on the Appearance of Turbidity in Sunflower Seed Oil, *J. Am. Oil Chem. Soc. 63*:1360–1363 (1986).
10. Brimberg, U.I., and I.C. Wretensjo, Rapid Method for Determination of Wax in Sunflower Seed Oil, *Ibid. 56*:857–860 (1979).
11. Rivarola, G., M.C. Añón, and A. Calvello, Crystallization of Waxes During Sunflower Seed Oil Refining, *Ibid. 62*:1508–1513 (1985).
12. Herrera, M.L., Crystallization Behavior of Hydrogenated Sunflower Seed Oil: Kinetics and Polymorphism, *Ibid. 71*: 1255–1260 (1994).
13. Herrera, M.L., and K. Sato, Kinetic Study of Crystallization of Hydrogenated Sunflower-seed Oil, *inform 5*:553 (1994).
14. Petruccelli, S., and M.C. Añón, Crystallization of Waxes in Sunflower Seed Oil: Effects of an Inhibitor, *J. Am. Oil Chem. Soc. 68*:684–686 (1991).

[Received September 17, 1999; accepted July 24, 2000]