Rapid Method for Determination of Wax in Sunflowerseed Oils

U.I. BRIMBERG and **I.C. WRETENSJÖ**, Food Division, Process Laboratory, Alfa-Laval AB, Tumba, Sweden

ABSTRACT

A rapid estimate of wax content in sunflower seed oil may be obtained by heating the oil to 130 C and subsequent cooling in ice for 10 to 15 min. Microcrystalline waxes are then formed. They are quantified by measuring increased turbidity with a sensitive turbidity meter. Calibration is made with thoroughly dewaxed oil to which known amounts of wax are added, and the turbidity increase is determined after 10 minutes at 0 C. The method has been compared with modified cold test, comprising visual inspection after 24 hours at 0 C and after 5 days at 20 C. The rapid tubidimetric method is superior to cold test in predicting the tendency of oils to precipitate small amounts of wax after long-time storage.

INTRODUCTION

In connection with the development of the Alfa-Laval refining-dewaxing process for sunflower seed oil, a rapid method for checking the dewaxing result was needed. Usually, the cold stability of the dewaxed oil is determined by a modified cold test, where the oil is prepared according to the AOCS method (1). The oil is kept at 0 C for 24 hr and then for 3–5 days at room temperature (20 C) before inspection of oil clarity. Several days are needed before results are obtained, and for process control a more rapid method was desirable.

The determination of waxes in sunflower seed oil, especially after dewaxing, is difficult due to the low wax concentration. In crude sunflower seed oils, the wax concentration can be 0.02–0.3% (2), but after dewaxing the remaining wax is in the range 0–150 ppm (3).

A gravimetric method including extraction in a special equipment was published by Ostric-Matijasevic (2). This method gives good results with crude oils, but fails with dewaxed oils. Morrison and Robertson (3) presented a method based on the isolation of the unsaponifiable material, including the wax. The different constituents of the unsaponifiable material were then separated by a gas liquid chromatographic method, and the amount of wax was calculated. This very informative method is not rapid enough for process control. Caupeil (4) has developed a rapid method, where the microcrystalline waxes formed upon cooling in an ice bath are accurately recorded with a sophisticated apparatus. However, the apparatus seems too complex for control use in the factory. We have therefore developed a simpler and more rapid method for the determination of wax in sunflower seed oil.

MATERIALS AND METHODS

Principle of the Method

The microcrystalline waxes, which form when a warm, wax-containing oil is rapidly cooled to 0 C, are not visible but can be recorded by means of a sensitive turbidimeter. A rapid determination can thus be made, and can be included as part of the usual cold test.

Sunflower oil, refined, but not necessarily bleached, is heated to 130 C to remove traces of moisture and completely melt all wax crystals initially present in the sample.

The filtration of the oil as prescribed in the AOCS method (1) must be made with the hot oil, i.e., immediately after the heating to 130 C. The hot oil is filtered in a warm Büchner funnel through a filter paper, retaining fine grain precipitates (Munktell No. 20H, Whatman No. 5). The filtration can be omitted for clear, bleached oils. The hot oil is poured into a clear Pyrex glass bottle (diameter 4 cm), and the turbidity of the hot oil is quickly measured in the Haze Meter, type UKM1 e (Radiometer, Copenhagen). The bottle is then placed in crushed ice for 10 min, and the turbidity is measured again. The difference in turbidity, ΔT, is a measure of the wax content of the oil. If desired, the bottle can then be kept for 24 hr in ice and for 3–5 days at 20 C and be inspected for oil clarity.

Calibration

A thoroughly dewaxed sunflower seed oil was produced in the laboratory by refining a Yugoslavian oil containing 4.1% of FFA. One portion of the oil was neutralized, washed, and dried. (The oil contained 15 ppm of soap and 0.5 ppm of phosphorus.) Another portion of the oil was refined as above and then bleached with 1% bleaching earth.

The bleached and unbleached oils were cooled slowly to room temperature and then placed for 4 days in a refrigerator. The oils were adjusted at 10 C and filtered through a layer of Celite 545 in a Büchner funnel kept at 10 C.

Pure wax to be used for calibration was prepared by de-oiling oily wax obtained from a factory, where refined sunflower seed oil was dewaxed by winterization. The deoiling was accomplished by precipitation of the wax in 10 times its volume of petroleum ether (b.p. 40–60 C). The mixture was kept over night in the refrigerator. The wax and remaining filter aid was filtered and washed with cold petroleum ether (b.p. 40–60 C).

The wax was heated to evaporate the petroleum ether and centrifuged at 100 C to separate the filter aid. The clear wax phase was drawn off by use of a warm pipette. The de-oiled wax had a melting point of 75.6 C.

A solution containing 1% by weight of wax in dewaxed oil was prepared as a stock solution. By further dilution, solutions containing 5, 10, 15, 20, etc., ppm of wax were prepared.

The solutions were heated to 130 C and transferred to a Pyrex glass bottle, which was sealed with a rubber stopper with turnover flange. A ΔT was measured after different times in an ice bath.

RESULTS AND DISCUSSIONS

Figures 1 and 2 show that after 10 minutes the precipitation of wax proceeds much more slowly than during the first minutes. The aim being a rapid method, we chose to take the turbidity difference after 10 minutes as a measure of the wax content. At high wax concentrations, there was a tendency to form air bubbles, which was more pronounced with the bleached oil, and the air bubbles caused peaks in the curves.

The thoroughly dewaxed oil with no added wax showed some small increase in turbidity, ΔT_O, which might to some extent be caused by coloring material in the oil, as the value was higher in the unbleached oil. However, there was probably also a very small amount of wax dissolved at 10 C (the dewaxing temperature) precipitating at 0 C in the test.

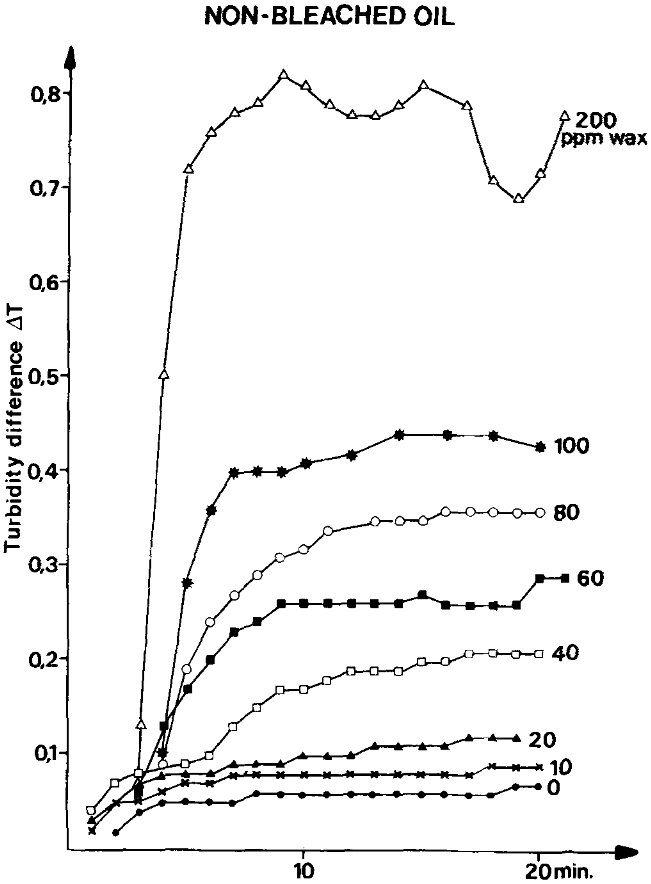

FIG. 1. Increase in turbidity with time at 0 C, nonbleached oil with added wax: 0, 10, 20, 40, 60, 80, 100, and 200 ppm.

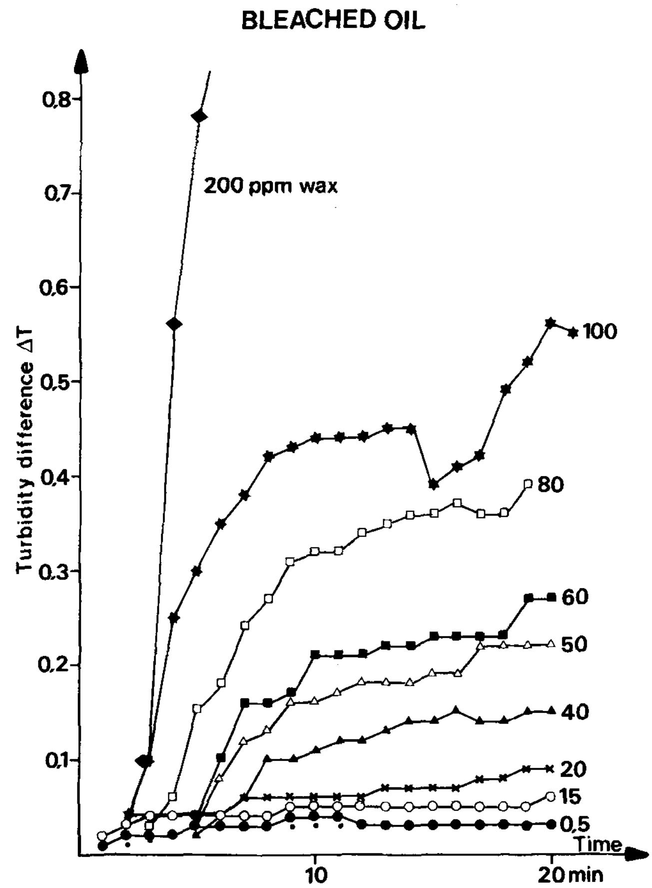

FIG. 2. Increase in turbidity with time at 0 C, bleached oil with added wax: 0, 5, 15, 20, 40, 50, 60, 80, and 100 ppm.

The turbidity increase corresponding to the added wax is the measured increase, ΔT, minus ΔT_O.

Table I gives the turbidity difference at 10 minutes, ΔT, for the different amounts of added wax and the corrected values, $\Delta T - \Delta T_O$. These corrected values are plotted against the added wax in Figure 3.

Strictly, the amount of wax determined in this way is not the total amount, as the wax dissolved at 10 C in the calibration oil ought to be added. If there were no contribution from coloring material, $\Delta T_O = 0.05$ for an unbleached oil corresponds to 20 ppm wax, and so does $\Delta T_O = 0.03$ for a bleached oil. Thus, 20 ppm wax is the maximum amount to be added. The amount of wax in an oil, thoroughly dewaxed at 10 C, could be determined with another method, e.g., according to Morrison and Robertson (3). This amount is likely to be rather constant for different sunflower seed oils.

The Haze Meter readings in Table I are given in duplicate pertaining to two calibration runs with the same oils on different occasions, about two months apart. These oils were not protected from light during storage, and as a result the color of the oils faded, and the turbidity of the hot oils decreased. The turbidity of the unbleached oil approached the initial turbidity of the bleached, fresh oil. However, the measured ΔT–after 10 min–was not affected within the accuracy of the determination. This shows that the color effect is greatly eliminated by taking the different ΔT.

The calibration curves for the bleached and nonbleached oils do not quite coincide. Up to ca. 200 ppm of wax, the calibration curve consists of two straight lines with a change of slope at ca. 40 ppm of added wax. Higher amounts of added wax, >200 ppm, gave less reproducible values, and in this range only a rough estimation of the wax content can be obtained.

Comparison with Visual Inspection

Table I also shows the appearance of the calibration liquids after storage in the refrigerator for 3 days at 6–8 C. The appearance of the oils is described as brilliant or clear, brilliant meaning perfectly transparent, clear meaning no detectable haze. The color of the oil does not affect this visual impression within the normal range of color of sunflower seed oils, although the Haze Meter indicates a higher turbidity in an oil of a stronger color.

After storage for 3 days at 6–8 C, there was a visible difference between 0 and 5 ppm of added wax, but only by direct comparison of the two samples in very sharp light. At 15 ppm of added wax, a few thin wax flocks could be seen which increased in size and number with increasing wax concentration.

Turbidity Measurement and Cold Test

Table II shows data from tests with some dewaxed oils. The turbidity measurement was supplemented with cold test inspection after 24 hr at 0 C and after subsequent storage for 5 days at 20 C.

Some bleached oils, notably samples 1, 3, and 8, showed ΔTs of 0.03–0.04 after 10 min at 0 C and remained clear after the complete cold test. A ΔT value of 0.03–0.04 corresponds to an amount of 0–5 ppm added wax according to the calibration curve, assuming ΔT_O to be 0.03.

Nonbleached oils that were clear after the cold test, samples 5 and 7, showed turbidity differences of 0.06–0.09 after 10 min at 0 C. Thus, according to the calibration curve for nonbleached oil with $\Delta T_O = 0.06$, wax contents

TABLE I

Turbidity Measurements for a Thoroughly Dewaxed Sunflower Seed Oil with Increasing Amounts of Added Wax

Added wax (ppm)	Nonbleached oil: Haze Meter reading 0 min	Nonbleached oil: Haze Meter reading 10 min	Nonbleached oil: ΔT	Nonbleached oil: $\Delta T - \Delta T_o$[a]	Bleached oil (1% Tonsil): Haze Meter reading 0 min	Bleached oil (1% Tonsil): Haze Meter reading 10 min	Bleached oil (1% Tonsil): ΔT	Bleached oil (1% Tonsil): $\Delta T - \Delta T_o$[b]	Appearance after 3 days at 6–8 C
0	0.36 0.21	0.42 0.25	0.06 0.04	0.00	0.21	0.24	0.03	0.00	Brilliant.
5	---	---	---	---	0.23	0.27	0.04	0.01	Clear, but not brilliant.
10	0.37	0.45	0.08	0.03	---	---	---	---	Clear, but not brilliant.
20	0.36	0.46	0.10	0.05	0.19	0.25	0.06	0.03	Some thin wax flocks.
40	0.36 0.20	0.53 0.34	0.17 0.14	0.10	0.23	0.34	0.11	0.08	A lot of thin wax flocks.
60	0.40 0.20	0.66 0.41	0.26 0.21	0.18	0.20 0.18	0.43 0.39	0.23 0.21	0.19	A lot of wax flocks.
80	0.34 0.23	0.67 0.55	0.33 0.32	0.28	0.21	0.53	0.32	0.29	Wax flocks and haze throughout the oil.
100	0.36	0.77	0.41	0.36	0.20 0.20	0.63 0.64	0.43 0.44	0.49	Dense wax flocks.
200	0.34 0.21	1.10 1.02	0.76 0.81	0.74	0.26 0.20	1.20 1.14	0.94 0.94	0.91	Many dense wax flocks.
300	0.22	1.65	1.43	1.38	0.33	1.90	1.57	1.54	Many dense wax flocks.
400	0.39	2.45	2.06	2.01	0.23	2.7	2.47	2.44	Completely cloudy.
500	0.37	3.7	3.33	3.28	0.22 0.18	3.8 3.1	3.6 2.9	3.4	Completely cloudy.

[a]$\Delta T_o = 0.05$.
[b]$\Delta T_o = 0.03$.

would be ca. 0–11 ppm.

The formation of microcrystalline waxes detectable in the Haze Meter is not finished after 10 min. After 24 hr at 0 C, a higher turbidity is usually obtained, as shown in Tables II and III. This formation of microcrystals is to some extent influenced by substances in the oil, as evidenced by the fact that the calibration curves for the bleached and nonbleached oils do not quite coincide. There might be an influence on the size, and hence on the number of the microcrystals, perhaps also on the formation rate.

The first appearing microcrystalline waxes, detectable in the Haze Meter, but not by the eye, form visible aggregates with time when the amount of wax is above a certain limit. Data in Table I indicate that this limit is ca. 10 ppm of added wax. The aggregation is a very slow process and seems to be slower in a nonbleached oil than in bleached oils. In some cases a dewaxed oil was still clear after 24 hr at 0 C and then 5 days at 20 C, yet some thin wax flocks precipitated on further storage at a low room temperature, e.g., at 15 C.

In order to accelerate the flock formation in oils, which were clear after the modified cold test, we placed the bottles with the samples from the modified cold test for a second time in ice for 24 hr, determined the turbidity and inspected the oil clarity. The bottles were then kept at 15–20 C for 1–5 days. In this way a kind of temperature conditioning of the oil sample was obtained which usually promotes crystallization. Of course, the time 5 days at 20 C was longer than necessary for conditioning, but we wanted to combine it with inspection of oil clarity after 5 days. Samples from some dewaxing test runs were treated in this way, and the results are given in Table III.

The samples in test run C were further kept for a long time at 15 and 20 C. After 1–2 months, traces of wax precipitated as a few very thin wax flocks or a slight haze at the bottom of the bottle in all 19 samples. Thus, the oil appearance after the second cooling to 0 C gave an indication of the oil clarity after long-time storage. This was, however, already indicated by the ΔT after 10 min at 0 C, which was 0.10, corresponding to 20 ppm of added wax, the limit being ca. 10 ppm, as mentioned above.

The standard deviation in the tests with nonbleached oil was 8–9 ppm wax and ca. 5 ppm wax in tests with bleached oil. About the same standard deviations are obtained from the calibration data.

Instead of making a calibration curve to determine the wax concentration, a limit for the turbidity increase after 10 min at 0 C can be determined by experience. Below this limit the oils show no tendency for wax precipitation after long-time storage at 15–20 C. According to our present experience with bleached sunflower oils, the limit is 0.03–0.04 measured in the Haze Meter. For nonbleached oils, this study suggests it is somewhat higher, ca. 0.08.

The wax crystallization is highly delayed or inhibited by impurities in the oil, such as phosphatides. Hence, a successful dewaxing can only be made with a refined oil. Morrison and Thomas (5) showed that winterization by crystallization in miscella before the refining did not remove but a small part of the waxes. Winterization after refining, however, removed the larger part of the waxes.

Because of these crystallization-inhibiting properties of

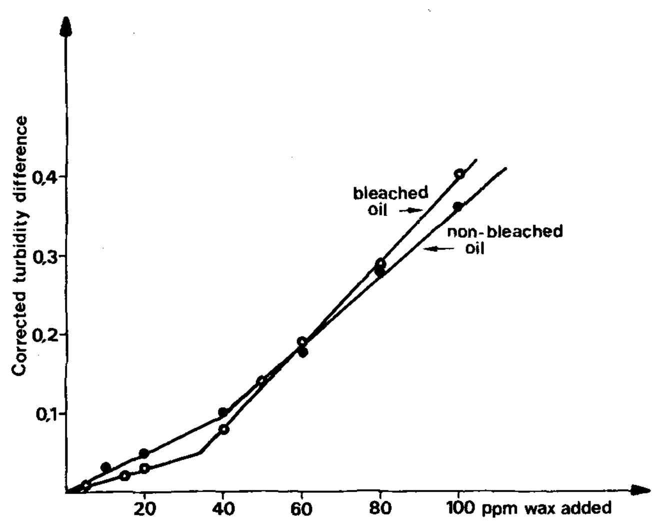

FIG. 3. Calibration curve. The corrected turbidity difference, $\Delta T - \Delta T_o$, is plotted against the amount of added wax (0–100 ppm).

TABLE II

Comparison of the Rapid Method with Modified Cold Test of Bleached and Nonbleached Sunflower Oil

Sample	Turbidity difference ΔT 10 min 0 C	Turbidity difference ΔT 24 h 0 C	Wax[a] ppm	Cold test appearance after 24 h at 0 C +	Cold test appearance after 5 days at 20 C	Remarks
1	0.04	0.17	5	Clear	Clear	The oil was bleached with 0.3% of Tonsil for 20 min at 80 C in vacuum. Not heated to 130 C.
2	0.18	0.35	53	Thin wax flocks	Thin wax flocks	The oil was bleached with 0.3% of Tonsil for 20 min at 80 C in vacuum. Not heated to 130 C.
3	0.03	0.18	0	Clear	Clear	The oil was bleached with 0.3% of Tonsil for 20 min at 80 C in vacuum. Not heated to 130 C.
4	0.16	0.19	50	Almost clear	Haze at the bottom of the bottle	The oil was bleached with 0.3% of Tonsil for 20 min at 80 C in vacuum. Not heated to 130 C.
5	0.06	0.20	0	Clear	Clear	Nonbleached oil
6	0.24	0.32	58	Clear	A few thin flocks	Nonbleached oil
7	0.09	0.19	11	Clear	Clear	Nonbleached oil. Average of triplicate analysis.
8	0.04	0.17	5	Clear	Clear	Sample 7, bleached with 1% Tonsil.

[a]ΔT_o = 0.03 for bleached oil and 0.06 for unbleached oil.

TABLE III

Comparison of the Rapid Method with Prolonged Cold Test of Bleached and Nonbleached Oils

Test run	Number of samples	Turbidity difference, ΔT[a] 10 min 0 C	Turbidity difference, ΔT[a] 24 h 0 C 1st time	Turbidity difference, ΔT[a] 24 h 0 C 2nd time	Wax ppm	Cold test appearance after 24 h 0 C 1st time	Cold test appearance after 5 d 20 C 1st time	Cold test appearance after 24 h 0 C 2nd time	Cold test appearance after 1–5 d 15–20 C 2nd time
A[b]	6	0.08 ± 0.026	0.19	0.19	11 ± 9	Clear	Clear	A few thin flocks	Clear[e]
B[b]	5	0.15 ± 0.025	0.22	0.19	40 ± 9	Clear	Clear	Wax flocks	Clear or slightly hazy[e]
C[c]	19	0.10 ± 0.022	0.22	0.24	20 ± 8	Clear	Clear	Slightly hazy	Clear[f]
D[d]	4	0.03 ± 0.008	0.14	0.10	0 ± 5	Clear	Clear	Clear	Clear[f]

[a]Average data and standard deviation.
[b]Nonbleached oil, ΔT_o = 0.05.
[c]Nonbleached oil, ΔT_o = 0.06.
[d]Bleached oil, ΔT_o = 0.03.
[e]One day 20 C.
[f]Five days 15 C.

phosphatides, the method presented here cannot be applied to crude oils.

REFERENCES

1. "Official and Tentative Methods of the American Oil Chemists' Society", Vol. 1, Third Edition, AOCS, Champaign, IL, 1964 (revised to 1970), Method Cc 11-53.
2. Ostric-Matijasevic, B., and J. Turkulov, Rev. Fr. Corps Gras 20:5 (1973).
3. Morrison, W.H., and J.A. Robertson, JAOCS 52:148 (1975).
4. Caupeil, J.E., Rev. Fr. Corps Gras 24:427 (1977).
5. Morrison, W.H., and J.K. Thomas, JAOCS 53:485 (1976).

[Received February 15, 1979]