

Rapid Methods for Predicting the Appearance of Turbidity in Sunflower Oil and Their Comparison with Cold Tests

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ABSTRACT: Two rapid turbidimetric methods have been applied to determine the content of wax in refined and refined-bleached sunflowerseed oils in the range of 0 to 120 ppm. Synthetic wax-oil mixtures were prepared to construct calibration plots. Turbidimetric results were compared with visual evaluation of these mixtures as well as of the industrial oils by an expert panel, during 2 wk, under cold-test (0°C) and tempering (15°C) conditions. Based on the standard AOCS cold-test, the visual turbidity threshold of these Argentine oils was under 40 ppm, a value significantly lower than that found in European cultivars. Also, while both types of industrial oils passed the cold-test, the refined oil developed cloudiness after tempering for 14 d, whereas the refined-bleached oil remained transparent. Because the calibration plots showed that the turbidity difference values, given by these fast methods, corresponded to wax contents that caused visible turbidity but fell into the non-sensitive region of the instrument, a simple standard addition technique was used to extend their sensitivity to these low wax contents. With this improvement, oils that contained more than 40 ppm of wax by fast turbidimetry, which can be expected to develop cloudiness on storage in the winter season, can be promptly identified, thus allowing any desired corrective action. *JAACS* 75, 363–370 (1998).

KEY WORDS: Cold-tests, sediment, sunflower oil, turbidimetry, waxes.

Turbidity in refined sunflowerseed oil, caused by the presence of high-melting lipid constituents, is a special problem whenever clear packaging is used to market this oil, because the appearance of a hazy cloud or sediment during shelf storage may render the product unacceptable to consumers.

The components that cause turbidity in vegetable oils are high-molecular-weight compounds, such as saturated triglycerides, waxes, free fatty acids and minor amounts of hydrocarbons, sterols and their esters, as well as fatty alcohols (1–4). In particular, sunflower oil sediment contains mostly wax esters, so that it is customarily designated as wax sediment or “wax” (5). In crude sunflower oils, the wax concentration can be 0.02–0.3%, but after winterization (or “dewaxing”), the remaining wax is in the range of 0 to 150 mg/kg (ppm) (6,7).

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The introduction of new sunflower varieties with high oil yields in the early 1980s required an increase in the efficiency of the winterization process because the wax content of the oil depends on the oil content of the sunflower seed, and the wax content in the hull of the new hybrids was 3.5 to 5 times greater than in earlier varieties (1,8–10).

It was soon realized, in connection with the development of new refining processes, that sensitive, reproducible, and rapid methods for checking dewaxing results were needed, other than gravimetric or visual tests (the so-called cold-tests). Because the microcrystals that form when a warm wax-containing oil is rapidly cooled are not visible but can be recorded by means of a sensitive turbidimeter, several research groups developed fast turbidimetric methods in the past decade and attempted to use these techniques to predict the tendency of oils to develop cloudiness or to precipitate a turbid sediment after long storage (8,11–13).

Unfortunately, vegetable oil refining processes include several steps besides winterization, such as elimination of gums, neutralization, bleaching, and deodorization. Also, because several sequences of these steps are possible in plant flowsheets, different crystallization conditions may occur in the winterization stage (e.g., temperature, residence times, and presence or absence of soaps and phospholipids), all of which may have an impact on its effectiveness (3,14–16). Therefore, development of reliable correlations between these fast turbidimetric methods and visual observations of long-term stability still seems unavoidable.

Likewise, it has become apparent that unbleached and bleached vegetable oils need different calibration curves, owing to influence of the size or the number of the microcrystals (or their formation rate) on the diverse long-term tendency of the oil to cloud or settle, so that correlations have to be established for both types of oils (11).

On these grounds, this work presents a systematic attempt to assess the capability of some of the available fast turbidimetric methods to predict the appearance of turbidity or cloudiness in nonbleached and bleached sunflower oil with a low-cost ratio turbidimeter, in conjunction with visual inspection by an expert panel under simulated warehouse long-term storage conditions as well as under typical cold-test conditions (17).

Sedimentation in oil can be viewed as a crystallization process and, as such, it proceeds *via* nucleation and crystal growth (18). Therefore, a standard addition technique was also used to check whether the sensitivity of the rapid turbidimetric techniques for determining the wax content in the low-ppm range could be improved.

MATERIALS AND METHODS

Samples of unbleached (hereafter, "refined") and bleached sunflower oils were obtained from an Argentinian oil processor. Stocks of both oils (10 L each) were heated to 130°C under gentle stirring to remove traces of moisture and completely melt all wax crystals initially present in the liquids. Immediately afterward, the oils were filtered through a medium-porosity filter paper in a warm, sleeved Büchner funnel, kept at 90°C by a heating fluid that was circulated from a thermostatic bath. Next, the oils were slowly cooled to room temperature and stored for further use (19).

Preparation of wax-free oils. A portion of each stock was used to obtain clear, wax-free materials, by following two alternative procedures. In the first procedure (D4), each oil was placed in a refrigerator at 4°C for 4 d; then heated to 10°C, and filtered through a layer of filter aid (Celite 545) in a Büchner filter kept at 10°C (11). In the second procedure, (D0), the oils were kept at 0°C for 3 d, prior to filtering as already described.

An initial attempt to obtain thoroughly dewaxed oils without filtering, by following the method of Rivarola *et al.* (14), was also made, but it had to be abandoned. Briefly, the oils were cooled to 0°C for 24 h and then centrifuged (10,000 × *g* for 60 min) at this temperature. A thin veil was then clearly distinguishable, but it could only be removed by filtration because every attempt to decant the liquid was unsuccessful.

Isolation of oil-free waxes. Pure wax to be used for calibration was prepared by extracting it from a filter cake obtained from the factory winterization process. The cake was first mixed with 10 vol of petroleum ether (b.p. 40–60°C) and kept overnight in a refrigerator at 4°C to precipitate the solute. Next it was filtered, washing it with cold petroleum ether (0°C). The deoiled filter cake (wax + filter aid) was then placed in a Soxhlet apparatus to extract the wax with petroleum ether (11). After evaporation of the solvent by vacuum distillation in a rotary evaporator, the wax obtained had a melting point of 73°C (20), and it showed a single narrow melting endotherm at around 74°C when heated in a differential scanning calorimeter at a heating rate 10°C/min (Mettler DSC-30, Hightstown, NJ).

Preparation of calibration wax–oil mixtures. Series of calibration wax–oil mixtures were prepared by mixing the dewaxed oils with known amounts of wax on a weight-to-weight basis (w/w). The mixtures were heated to 130°C under gentle stirring, transferred to dark bottles, and stored at 23°C. Table 1 lists the full set of calibration mixtures, together with the codes of the dewaxed oils used (e.g., R-D4 stands for refined-dewaxed at 4°C).

TABLE 1
Wax Content (ppm) of the Calibration Wax–Oil Mixtures

R-D4	Type of oil ^a		
	R-D0	RB-D4	RB-D0
0.0	0.0	0.0	0.0
13.7	12.9	14.2	12.4
19.8	24.3	25.4	22.1
45.0	41.9	41.6	41.2
50.9	50.4	48.8	54.8
54.6	65.2	68.6	63.9
93.4	75.1	77.4	85.4
110.0	124.8	115.8	108.1

^aThe refined (R) and refined-bleached (RB) sunflower oils were subjected to dewaxing prior to their mixing with pure wax. The codes D4 and D0 stand for previous dewaxing procedures of the oils, after keeping them for 4 d at 4°C (D4) or for 3 d at 0°C (D0), prior to their filtering at 10°C.

These synthetic mixtures can be considered good approximations to sediment-forming sunflower oils. Indeed, as Liu and co-workers showed (5), sunflower oil sediment is largely constituted by wax esters (sometimes more than 99% of the total), unlike canola oils where the mass fraction of polar compounds, mostly saturated fatty acids, may represent up to 20% of the sediment.

Turbidimetric measurements. Two different measuring protocols were chosen and compared.

In Method BW [after Brimberg and Wretensjö (11)], a portion of approximately 150 mL sunflower oil was heated to 130°C to remove traces of moisture and completely melt all wax crystals present, as prescribed in the AOCS method (17), and then immediately filtered in a warm Büchner funnel through a medium-porosity filter paper. The first 20–30 mL were discarded. The hot oil was again heated, removed from the heat source immediately when it reached 130°C, and poured into a sample cell, where a first reading of its turbidity was done (T_1). Next, the sample cell was removed from the instrument and placed in an ice bath for 10 min, after which the turbidity was measured again (T_2). The difference in turbidity, $T_2 - T_1$, gives a measure of the wax content of the oil. Prior to obtaining the second turbidity readings, the sample cells were set at room temperature for 2–3 min, carefully rinsed with alcohol, and wiped with tissue paper to eliminate streaks or smudges.

In Method RCT (after the "Rapid Chill Method" of the DeSmet Laboratory, Edegem, Belgium), the sample cell was placed in a constant-temperature bath at 5.5°C for 1 h right after the first turbidity reading. Then, the second reading (T_2) was done. Special care was taken to ensure that the sample cell was set in the bath with the coolant level equal to its oil level.

To construct reference (calibration) curves, aliquots of the calibration wax–oil mixtures of the refined and refined-bleached oils were subjected to these two measuring protocols, with three replicates for each mixture. A Hach ratio turbidimeter, model XR (Hach Company, Loveland, CO) and standard Hach (30 mL) round Pyrex cells were used. The cells were previously adjusted to obtain maximal signals by rotating them inside the sample holder, and marked as indicated

by the manufacturer. The range of the instrument was set at 20 NTU. Measurements were done under a stream of dry nitrogen.

Because it is known that, whenever a piece of fiber is floating around in the oil, the number reading changes rapidly on the instrument, care was taken to wait until the turbidity values became stable before recording them. Also, handling of the sample cells was extremely cautious to minimize shaking or disturbing them because, at high wax concentration, there is a tendency for air bubbles to form, which may cause peaks in the curves.

A stock solution with 86.8 ppm of wax was also prepared from refined sunflower oil, dewaxed at 0°C (R-D0), to represent the wax-free starting material. Aliquots of this stock solution were added to the set of calibration mixtures that corresponded to this oil (in a 1:1 w/w ratio) and were used to construct a new reference curve to check whether the ability of the rapid turbidimetric techniques for determining the wax content in the low-ppm range could be improved by means of a classical standard addition technique. Only method BW was tested in this fashion.

Comparison with visual inspection. The physical appearance of aliquots of each of the four series of standard wax–oil mixtures, as well as of the refined and refined-bleached sunflower oils, was evaluated by a five-member panel.

The panel members were asked to describe the visual appearance of the oils as “transparent” or “clear.” Transparent meant perfectly brilliant (17), that is, no turbidity could be observed. If haze or flocs were present, the panel was also asked to describe the shape and size of the flocs or wax agglomerates and/or to report whether any sediment had settled at the bottom of the flasks.

The oils underwent two different procedures: (i) AOCS cold-test (17), stored at 0°C, with readings done after 5.5 h, then after 1, 2, 3, 7, and 14 d; and (ii) tempering at 15°C in a constant-temperature water bath, with inspection of the samples after 1, 2, 3, 7, and 14 d. The selected temperature is the typical condition in our local warehouses during the winter.

In both procedures, sealed transparent glass bottles (125 mL) were used, and each bottle was assigned a random code number; sample duplicates were used. The comparisons were always done against a backlighted dark background.

RESULTS AND DISCUSSION

Turbidity measurements. Figures 1 and 2 show typical reference curves obtained with the series of calibration wax–oil mixtures of refined and refined-bleached sunflowerseed oil, after using both of the dewaxing procedures described above. The values obtained with Method BW were always more reproducible and gave smoother plots. They also were much more stable. That is, when the oil samples were kept at 0°C for 10 min prior to the second turbidity reading (T_2), the instrument did not show readout “excursions,” and stable T_2 values could always be recorded. When Method RCT was employed instead, and the second turbidity reading was taken

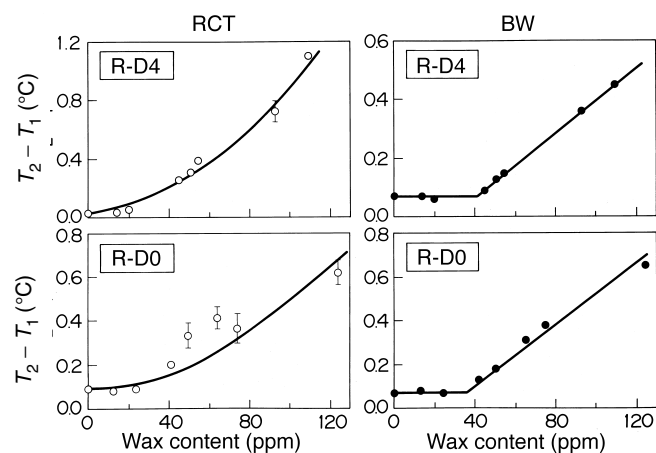


FIG. 1. Calibration plots (turbidity difference readings vs. amount of added wax) obtained with synthetic wax/refined oil mixtures with the rapid chill test (RCT) and the Brimberg–Wretensjö (BW) rapid turbidimetric methods. The first turbidity readings (T_1) are taken while the oil is warm, and the second readings (T_2) are taken after placing the samples at 5.5°C for 1 h (method RCT) or at 0°C for 10 min (method BW). The codes D4 and D0 stand for previous dewaxing procedures of the oils, after keeping them for 4 d at 4°C (D4) or for 3 d at 0°C (D0). Presented values are means of three replicates. The full range of values is shown only if the coefficient of variation exceeded 15%.

after keeping the samples at 5.5°C for 1 h, unstable readings were obtained on some occasions. Despite this shortcoming, though, the RCT method always gave equal or larger turbidity difference readings ($T_2 - T_1$) than the BW method, probably because the crystallization time is longer in the former procedure (1 h instead of 10 min), which allows for improved crystal growth (16).

It is also obvious from these figures that there are no appreciable increases in turbidity difference readings with either turbidimetric method, for both types of oil, when the wax content is in the 0 to 40 ppm range.

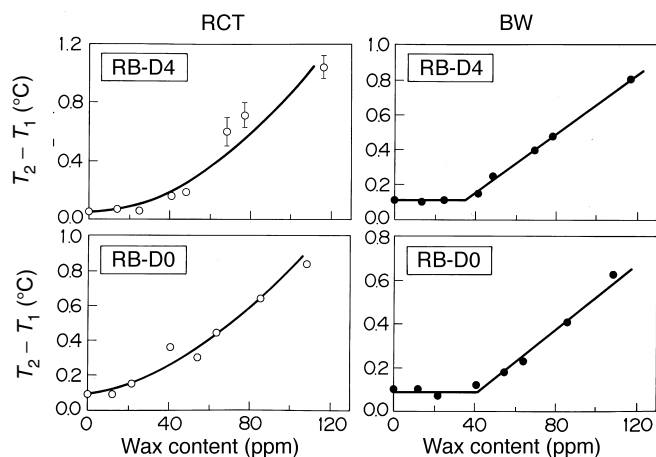


FIG. 2. Calibration plots (turbidity difference readings vs. amount of added wax) obtained with synthetic wax/refined-bleached oil mixtures with the RCT and BW turbidimetric methods. Presented values are means of three replicates. The full range of values is shown only if the coefficient of variation exceeded 15%. For abbreviations see Figure 1.

Oil sediment crystallization and melting processes are complex and depend on cooling rate, coolant temperature, wax concentration, and contents of natural inhibitors (mostly phospholipids) of the crystallization process. The degree of supercooling increases when the wax content decreases, and it is higher when the cooling rate is larger (14,16,18). Crystal formation (nucleation) times decrease at lower coolant temperatures, but they tend to equalize at high cooling rates (14).

High cooling rates are used in the protocols of both turbidimetric methods used in this work (sample tubes are immersed in constant-temperature baths); so, the degree of supercooling is high (16). Consequently, many nuclei are formed, and crystals may be too small to be detected when the wax content is less than about 40 ppm.

The temperature used to thoroughly dewax the oils to make the synthetic calibration mixtures was a relevant parameter. For the refined-bleached oil, the size of turbidity difference readings ($T_2 - T_1$) of the reference curves was consistently larger by both turbidimetric methods when the oil used to prepare the series of calibration solutions had been dewaxed at 4°C rather than at 0°C (Fig. 2). For the refined oil, though, only method RCT gave larger turbidity differences, whereas about the same results were obtained by either D4 or D0 wax-free oils with method BW (Fig. 1).

Indeed, on the basis of earlier data obtained with sunflower oil, the possible occurrence of these “matrix effects” was not entirely unexpected (8); so, our procedures to obtain wax-free oils (D4 and D0) allowed for at least 3 d of chilling prior to the filtering step in either procedure. Recently, Przybylski *et al.* (4) reported that the rate of sediment formation of refined-bleached-deodorized (RBD) canola oil (measured by conventional spectrophotometry) increases significantly when the oil is stored in a refrigerator at 2°C rather than at 6°C, but that after three consecutive days at either temperature, their transmittance readings became stable and similar. However, as properly pointed out by these authors, in addition to the concentration of high-melting crystallizing compounds, other endogenous constituents can influence the metastability of the oil–sediment system (4).

On these grounds, we believe that it is more appropriate to prepare stocks of “thoroughly dewaxed” refined or refined-bleached oils, to make calibrating wax–oil mixtures and construct reference/calibration curves, by placing the starting materials at 4°C for 4 d inside a refrigerator (procedure D4 above), instead of using a more cumbersome ice-water bath at 0°C (procedure D0). Incidentally, Liu and coworkers (18) found that the optimal temperature for short-term sediment concentration and development in canola oil, in terms of nucleation and crystal growth, is about equal, 5°C, but this might be just coincidental because canola oil sediment contains large amounts of high-melting saturated triglycerides (3).

As for the turbidimetric methods themselves, we believe that these combined results suggest that method BW is preferable to the RCT method because it is not only faster but also more precise (CV <10%), which more than compensates for the lower turbidity difference readings that it produces.

Turbidimetric measurements of the wax content in samples of the as-received refined and refined-bleached sunflowerseed oils were also performed, during four consecutive days, by both the BW and the RCT methods. Table 2 details the average values obtained. From these measurements it follows that the wax contents of both the refined and refined-bleached oils were less than about equal to 40 ppm, but clearly no precise estimates could be obtained with either method because the calibration curves became flat lines whenever the wax content was under 40 ppm.

Because sedimentation in oil proceeds *via* nucleation and crystal growth, a classical standard addition technique was then used, as detailed in the Materials and Methods section, to check whether the ability of these rapid turbidimetric methods for determining the wax content in the low-ppm range (and hence their predictive power) could be improved by “seeding” nucleation centers.

As a qualitative sensitivity analysis of Figures 1 or 2 readily shows, to maximize the discriminating capability in the 0 to 80 ppm range, it is convenient to use as the additive, for either refined or refined-bleached sunflower oils, a stock solution of a standard wax–oil mixture that contains about 80 ppm wax. Then, a new reference curve can be constructed by simply adding aliquots of this standard wax–oil solution to the series of calibrating mixtures in a 1:1 w/w ratio.

To test the concept and exemplify matters, the set of calibrating mixtures corresponding to the refined sunflowerseed oil dewaxed at 0°C (R-D0) was mixed 1:1 w/w with a stock solution that contained 86.8 ppm of wax, as detailed in the Materials and Methods section. Figure 3 depicts comparative results of calibration curves obtained by using the conventional BW method and the improvement achieved by using the standard addition technique, for the refined sunflower oil (R-D0). Clearly, this improved technique allows good discrimination of the wax contents in the 0 to 50 ppm range.

The excellent reproducibility of method BW is also remarkable because the turbidity difference readings ($T_2 - T_1$) of the stock solution with 86.8 ppm of wax fell almost exactly on the straight line of the reference curve that had been built a fortnight before (Fig. 3). A sample of the refined oil received from the factory was also diluted with the stock solution in the same fashion (1:1 w/w) and tested. The assay gave 27 ± 5 ppm of wax.

TABLE 2
Wax Content of the Industrially Refined and Refined-Bleached Sunflower Oils^a Given by the BW and the RCT Turbidimetric Methods

Type of industrial oil	Wax content (ppm) ^b	
	RCT	BW
Refined	<40	45 ± 5
Refined and bleached	<40	<40

^aAs given by the BW and the RCT turbidimetric methods. Abbreviations: BW, Brimberg and Wretensjö method (Ref. 11); RCT, rapid chill method (DeSmet Central Laboratory, Edegem, Belgium).

^bAverage values of measurements (three replicates) taken during four consecutive days ± standard deviation.

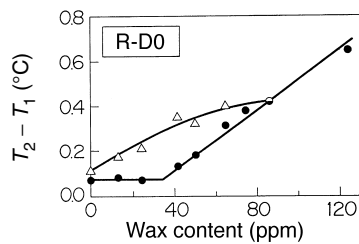


FIG. 3. Comparison of calibration plots (turbidity difference vs. amount of added wax) obtained with synthetic wax/refined oil mixtures by the BW turbidimetric method: ●, conventional method (undiluted samples); △, Improved sensitivity in the low-ppm range, by using a standard addition technique (calibration samples were diluted 1:1 w/w with a standard stock that contained 86.8 ppm wax); ○, standard stock, neat. Only average values are shown because the coefficient of variation was always less than 10.5%. For abbreviations see Figure 1.

Visual inspections. Tables 3–6 show the results of the whole set of observations of the expert panel on the visual appearance of the complete set of calibration samples of the refined and refined-bleached oils, as well as of the as-received factory samples, after storage under cold-test conditions and tempering at 15°C, respectively. The patterned areas inside each box are proportional to the number of opinions in the panel.

In either storage condition, the visual appearance of the series of calibrating mixtures of the refined vs. the refined-bleached oil was quite similar, albeit the latter series were always slightly better in the sense that they developed sediment or cloudiness later or for higher wax contents.

The observed wax flocs were small in size, separate, spherical, and dense in the samples tempered at 15°C, for both refined and refined-bleached types. These flocs tended to settle quite easily; the sediment had a powder-like appearance.

On the contrary, the wax flocs were larger and thin, with loose, tenuous borders when the samples were subjected to the cold-test at 0°C. In successive days, these flocs clustered. Also, while isolated flocs were observed at the earlier stages of the cold-test, more flocs became apparent with time, and eventually they occupied the whole bottle. This process was more pronounced at higher wax contents.

These noticeable differences between the two treatments were expected because at higher temperatures, vegetable oils are less viscous, thus enabling the faster diffusion of wax molecules and promoting crystallization (22,23).

A more detailed analysis of the observations of the visual inspection panel indicates the following.

For the cold-test at 0°C (Tables 3 and 4), (i) both the industrially refined and refined-bleached oils exceed the AOCS requirement (transparent appearance after 5.5 h at 0°C). (ii) The visual appearance from day to day of either industrial oil (shown in the last row of the tables) can be ranked between that of the calibration wax–oil mixtures that contain wax in the range of 20 to 40 ppm. (iii) In general, the series of calibration mixtures, prepared from oils previously dewaxed at 4°C (R-D4 or RB-D4), show a slightly poorer performance than those obtained from dewaxing at 0°C (procedure D0), and develop cloudiness earlier or at lower wax content. This may be due to the fact that the process of dewaxing

TABLE 3
Visual Appearance of Industrial Refined Sunflower Oil and of Its Wax–Oil Calibration Mixtures After Storage at 0°C (“Cold-Test”)^a

	Wax added (mg/kg)	AOCS cold test (5.5 h)	Cold test appearance after (days)				
			1	2	3	7	14
Oil dewaxed at 4°C	0.0	4a,1b	4a,1b	5a	1a,3b,1c	1a,2b,2c	1b,1c,2d,1e
	13.7	4a,1b	5a	3a,2b	2b,2c,1d	5d	5d
	19.8	5a	4a,1b	2b,2c,1d	5d	4d,1f	5d
(R-D4)	45.0	1a,4b	1b,4c	1b,2c,2d	5d	5d	5d
	50.9	2a,2b,1c	4b,1c	1b,2d,2e	5d	5d	4d,1f
	54.6	2b,2c,1d	3b,1c,1d	3d,1e,1f	4d,1e	5d	5d
	93.4	1a,3b,1c	4c,1d	1a,1c,1d,2e	5d	5d	5d
	110.0	3c,1d,1e	2d,3e	1c,1d,2e,1f	1d,4e/2s	1d,3e,1f/2s	5d/1s
Oil dewaxed at 0°C	0.0	5a	5a	4a,1b	4a,1b	3a,2b	2a,3b
	12.9	5a	4a,1b	3a,2b	2a,2b,1c	5d	5d
	24.3	2a,3b	1a,3b,1c	2b,2c,1d	1a,3b,1c	5d	5d
(R-D0)	41.9	1a,3b,1c	5b	2c,2d,1e	3d,1e,1f	1c,4d	5d
	50.4	1a,3b,1c	1a,3b,1c	1b,3c,1d	5d	5d	5d
	65.2	5b	2b,3c	2d,3e	1c,1d,2e,1f	5d	5d
	75.1	1a,3b,1c	2b,2c,1d	1c,1d,3e	2d,2e,1f	5d	5d
	124.8	1c,2d,2e	2c,3d	2d,3e	4d,1e	4d,1e	5d
Industrial oil		5a	3a,2b	1a,2b,1c,1d	1a,3b,1c	4d,1e	5d

^aNumbers correspond to the relative weight (maximal value of 5) of each subjective description (average of two replicates) in the expert panel. The letter code is as follows: a, transparent (“brilliant”); b, clear, but not transparent; c, some wax flocs; d, numerous wax flocs; e, wax flocs and haze throughout the oil; f, completely cloudy; /s, sediment at the bottom. For letters c–e, the observed wax flocs were thin with loose, tenuous borders.

TABLE 4
Visual Appearance of Industrial Refined-Bleached Sunflower Oil and of Its Wax–Oil Calibration Mixtures
After Storage at 0°C (“Cold-Test”)^a

	Wax added (mg/kg)	AOCS cold test (5.5 h)	Cold test appearance after (days)				
			1	2	3	7	14
Oil dewaxed at 4°C	0.0	5a	5a	5a	5a	5a	5a
	14.2	5a	4a,1b	5a	4a,1b	1a,3c,1d	2a,2c,1d
(RB-D4)	25.1	3a,2b	2a,3b	2a,2b,1c	1a,1b,1c,1d,1e	1a,2c,2d	2a,1c,2d
	41.6	2a,2b,1c	1a,3b,1c	2b,3c	1c,2d,2e	5d	1c,4d
	48.8	1a,3b,1c	4b,1c	1b,1c,1d,1e,1f	1c,3d,1f	1c,4d	1a,1c,3d
	68.6	4b,1c	4b,1c	3d,1e,1f	1c,1d,2e,1f	5d	5d
	77.4	3b,2c	1b,3c,1e	1c,1d,2e,1f	1c,1d,2e,1f	1c,1d,2e,1f	1c,3d,1f
	115.8	2c,2d,1e	3b,2d	1b,1c,1d,1e,1f	1c,2d,2f	1c,1d,1e,2f	2e,3f
Oil dewaxed at 0°C	0.0	5a	5a	5a	5a	5a	5a
	12.4	3a,2b	5a	5a	4a,1b	1b,1c,3d	1a,3c,1d
	22.1	2a,3b	3a,2b	2a,2b,1c	1b,2c,2d	5d	1a,3c,1d
(RB-D0)	41.2	2a,3b	4b,1c	2c,2d,1e	5d	1a,2c,1d	1a,2c,2d
	54.8	1a,3b,1c	3b,2c	1b,2c,1d,1f	1b,2c,1e,1f	4d,1e	5d
	63.9	4b,1c	2b,1c,1d,1e	1b,2c,1d,1e	1c,2d,1e,1f	1d,4e	4d,1e
	85.4	4b,1c	3b,1c,1e	1b,1c,1d,1e,1f	1d,2e,2f	3d,2e	4d,1f
	108.1	2b,1c,1d,1e	5e	1c,2d,2e	1c,2d,2e	1d,3e,1f	2d,3e
Industrial oil		5a	3a,1b,1c	4b,1c	3b,2c	3b,2c	2c,1d,1e

^aFor footnote see Table 3.

by cooling for several days at the lower temperature leaves a lower residual amount of nuclei formation inhibitors [humidity, soaps, and/or phosphatides (15)]. Regardless, the usage of these wax–oil calibrating mixtures (i.e., starting from oils dewaxed after being kept for 4 d in a refrigerator at 4°C) yields a more conservative criterion to estimate whether these oils will eventually develop cloudiness or sediment in warehouses, aside from the simplicity of their preparation method already pointed out above.

Regarding the tempering procedure at 15°C (Tables 5 and 6), (i) again, both the industrially refined and refined-bleached samples showed visual turbidity that can be located between that of the calibrating mixtures in the range of 20 to 40 ppm. (ii) The calibrating series prepared from oils dewaxed at 4 or 0°C showed about the same performance; the former was slightly poorer.

This study shows that the visual turbidity threshold of refined or refined-bleached sunflower oils of Argentine culti-

TABLE 5
Visual Appearance of Industrial Refined Sunflower Oil and of Its Wax–Oil Calibration
Mixtures After Storage (Tempering) at 15°C^a

	Wax added (mg/kg)	Appearance after tempering during (days)				
		1	2	3	7	14
Oil dewaxed at 4°C	0.0	5a	5a	5a	5a	5a
	13.7	5a	4a,1b	5a	4a,1b	5a
	19.8	5a	5a	5a	5a	5a
(R-D4)	45.0	2a,3b	3c,2d	3c,2d/1s	5d/5s	5d/5s
	50.9	1a,1b,2c,1d	3c,2d/1s	2c,3d/3s	5d/5s	5d/5s
	54.6	3c,2d	5d/1s	5d/1s	5d/4s	5d/4s
	93.4	5d	4d,1e/1s	5d/4s	5d/5s	5d/5s
	110.0	5d	5d/3s	4d,1e/1s	4d,1e/1s	4d,1e/5s
Oil dewaxed at 0°C	0.0	5a	5a	5a	5a	5a
	12.9	5a	5a	5a	5a	5a
	24.3	5a	5a	4a,1b	4a,1c	1a,3c,1d/3s
(R-D0)	41.9	4a,1b	4c,1d	4c,1d	2c,3d/1s	1c,4d/4s
	50.4	3c,2d	2c,3d	3c,2d	1c,4d/4s	5d/5s
	65.2	5d	5d	5d	1c,4d/5s	5d/5s
	75.1	1c,4d	5d/1s	4d,1e/3s	5d/4s	5d/4s
	124.8	4d,1e	5d/5s	5d/3s	5d/5s	4d,1e/4s
Industrial oil		5a	5a	5a	5a	5c/1s

^aFor footnote see Table 3. For letters c–e, the observed wax flocs were spherical and dense.

TABLE 6
Visual Appearance of Industrial Refined-Bleached Sunflower Oil and of Its Wax–Oil Calibration Mixtures After Storage (Tempering) at 15°C^a

	Wax added (mg/kg)	Cold test appearance after (days)				
		1	2	3	7	14
Oil dewaxed at 4°C (RB-D4)	0.0	4a,1b	5a	5a	5a	5a
	14.2	5a	5a	5a	5a	5a
	25.1	5a	5a	3a,2c	2a,3c	3a,2c/2s
	41.6	4c,1d	1a,2c,2d	2c,3d	5d/2s	1c,4d/5s
	48.8	5c	1c,4d/3s	2c,3d/3s	1c,4d/4s	5d/5s
	68.6	2c,3d	5d/2s	2c,3d/2s	5d/5s	5d/5s
	77.4	1c,4d	5d/3s	5d/3s	5d/5s	4d,1e/5s
115.8	4d,1e/2s	1c,4d/4s	4d,1e/4s	1c,3d,1e/5s	4d,1e/5s	
Oil dewaxed at 0°C (RB-D0)	0.0	5a	5a	5a	5a	5a
	12.4	5a	4a,1b	4a,1b	4a,1b	5a
	22.1	4a,1b	4a,1b	5a	5a	4a,1c
	41.2	1a,1b,3c	5d	1c,4d/1s	5d/1s	5d/5s
	54.8	4c,1d	3c,2d	3c,2d	2c,3d/5s	5d/5s
	63.9	1c,4d	5d	1c,4d/2s	5d/3s	4d,1e/5s
	85.4	4d,1e	4d,1e/2s	5d/3s	5d/5s	4d,1e/4s
108.1	1c,4d	5d/2s	4d,1e/3s	5d/5s	4d,1e/5s	
Industrial oil		5a	5a	4a,1b	5a	4a,1c

^aFor footnote see Table 3. For letters c–e, the wax flocs were spherical and dense.

vars, by the standard AOCS cold-test (i.e., cooling to 0°C for 5.5 h), is under 40 ppm, a value significantly lower than that found by Turkulov *et al.* (8) in European cultivars (80 ppm), and also much lower than that of canola oils, which was found to be of about 200 ppm (18). Also, while both types of industrial oils can pass the AOCS cold-test satisfactorily, the refined oil may show poor performance by developing a sediment upon long-term warehouse storage at about 15°C, but the refined-bleached oil is likely to remain transparent.

These trends confirm earlier data that were obtained with sunflower oils (8,11,14), but they certainly should not be taken as general criteria by vegetable oil processors because, for instance, Liu *et al.* (16) have recently reported entirely different patterns of tolerance to sedimentation vs. storage temperature in canola oil.

This work shows that, with just minor modifications, the available turbidimetric methods for rapid determination of the wax content in these types of sunflower oils can give adequate estimates about their future shelf performance.

The calibration plots constructed with synthetic mixtures showed that the turbidity difference values given by these fast methods, corresponding to wax contents that can cause visible turbidity appearance, fell into the nonsensitive region of the instrument, and a simple standard addition technique can be used to boost their sensitivity for these low wax contents and enhance their predictive power.

With this improvement, oils that contain more than 40 ppm of wax by fast turbidimetry and that can be expected to develop cloudiness on storage in the winter season can be promptly identified. Thus, with an affordable turbidimeter, corrective action can be taken in the refining process, if needed, in shorter times, and avoids the time-consuming vi-

sual inspections by cold-test or tempering procedures. The technique may also be helpful, for quality-control purposes, to small-scale vegetable oil packers or retailers.

Combined results suggest that it is better to prepare calibration wax–oil mixtures by using dewaxed oils filtered at 10°C after 4-d storage in a refrigerator at 4°C. Measurements of the turbidity differences by method BW are more reproducible than those obtained by method RCT.

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