Evaluation of Cold-Test Methods for Screening Cloudy Canola Oils

Ingrid Botha and Rodney J. Mailer*

New South Wales Agriculture, Wagga Wagga Agricultural Institute, Wagga Wagga 2650, Australia

ABSTRACT: Cloudiness in canola (*Brassica napus*) oil is currently tested using methods that involve cooling the oil samples and observing them for the development of cloudiness. Alternative methods include the use of solvents to increase sensitivity or speed. We compared traditional temperature-only cold tests and solvent cold tests for canola oils which sediment at room temperature, only while refrigerated, or not at all. All oils tested passed the American Oil Chemists' Society cold test, and all but one heavily sedimenting oil passed a commercial refiner's cold test. The efficiency of the commercial refiner's test was improved by lowering the temperature from 15 to 7°C. Of the four solvents tested, acetone was the most effective in detecting cloudy oils. The most effective combination was a cold test using 70% cold acetone, observed after 6 h storage in an ice-water bath.

Paper no. J9682 in *JAOCS 78*, 395–399 (April 2001).

KEY WORDS: Canola oils, canola sediment, clouding, cold test, oil, salad oil, sediment.

Canola oils are generally clear, bright, and attractive to consumers. However, canola oils may become cloudy and form a precipitate during storage, a problem that has become more apparent in recent years. Salad oils, such as canola, are often refrigerated which further promotes cloudiness.

The substances which cloud canola oil are often referred to as "wax." The major components of the clouding material are either triacylglycerols (TAG) (1,2) or wax esters (3). Minor components may include hydrocarbons, free fatty acids, and alcohols, diacylglycerols, and phospholipids (1–3). Sediment formation in both canola and sunflower oils is dependent on temperature and the concentration of the components contributing to sedimentation (3,4). Long-chain wax esters have high melting points, which may cause them to crystallize and drop out of solution. Saturated TAG have been shown to have a role in the sedimentation of palm (5) and canola oils (2). Highly unsaturated palm oil was more resistant to clouding (5).

Winterizing, or cooling to promote the formation of crystals, is used to remove sediment from refined oil. Winterizing is expensive, and it is more cost effective to winterize only those batches of oil that are likely to sediment. However, cloud tests are not always accurate.

The American Oil Chemists' Society (AOCS) cold test (Cc 11-53) is one of several methods used to predict clouding behavior in oils although this test is often unreliable (4,6). Modifications to the AOCS test include longer storage times to allow greater sediment formation (3,4, 6,7). Suggested temperatures for testing include 0 (6,8), 2, and 5°C (3), and 13°C (4). The rate of cooling influences the form and size of crystals. Rapid cooling produces smaller crystals while slower cooling produces larger crystals (9). An oil containing many small crystals may pass a cold test while an oil with fewer, large crystals will fail. Some modified AOCS tests use lower initial heating temperatures such as 75 to 80°C (3). Hexane (3), acetone (3,10), and acetone/hexane (8) have been used, and cloudiness was evaluated visually or with a turbidimeter. The aims of this study were to compare published cold-test methods and to determine the most suitable method by comparing effects of storage temperature and solvent use.

*To whom correspondence should be addressed.
E-mail: rodney.mailer@agric.nsw.gov.au

EXPERIMENTAL PROCEDURES

Oil samples. Oil samples were commercial oils obtained from local supermarkets or refiners. These included 28 canola oil samples and 1 sunflower sample. Samples were grouped according to sedimenting behavior. They included refined, bleached, and deodorized canola oils, which sediment at room temperature (samples 1–7), refined canola oils with heavy sediment at 7°C (samples 8–12), canola oils with light sediment at 7°C (samples 13–17), and canola oils which do not sediment at all (samples 18, 19). The samples also included cold-pressed canola oils, which sediment when refrigerated (samples 20–24). The sedimentation behaviors of three canola oils (samples 25–27), at room temperature and with long-term storage, were not determined as they were modified, in the development of the test, immediately after purchase. Sample 28 was oil recovered from canola filter cake, used to refine canola oil. Sample 29 was sunflower oil, which sedimented at room temperature.

Cold-test methods. The AOCS Official Method Cc 11-53, and variations on it, were compared. Oils (samples 3–27) were heated to 130°C to remove moisture and crystals that may have been present, and then filtered. Bottles (200 mL) were filled to capacity and then placed in a 25°C water bath, followed by an ice-water bath for 5.5 h. Oils that remained visually clear after 5.5 h passed the cold test.

Commercial refiner's test. Samples 2–7, 9–14, 16–19, 21–24, and 29 were tested using a cold test used by a commercial refiner. This method, although details have been asked to be kept confidential, involved storing 500 mL oil at 15°C. Oils were visually checked for clarity after 24 and 48 h. Oil that remained clear for 48 h passed the test.

Effect of storage temperature. Lower temperatures than those that were used in the commercial refiner's test were used to screen samples 1–14, 16–19, 21–24, and 29. These samples were stored at 7°C for 7 d after storage at room temperature but without prior heating. Oils were examined visually over 7 d. Oils that remained clear at each observation passed the cold test, and oils that became hazy or sedimented failed. These samples previously stored at 7°C were again tested at 1–3°C for 7 d after storage at room temperature but without prior heating, and again examined for clarity.

Solvent-aided cold tests. All solvents were chromatography grade and were cold (–20°C), to get rapid results, when combined with oil samples. The solvent/oil combinations for each study were stored in an ice-water bath and observed regularly. Four solvents were compared for their abilities to hasten sediment formation. Hexane (3), acetone (3,10), petroleum ether (3), and acetone/hexane (85:15, vol/vol) (8) were chosen because of their demonstrated use in either cold tests or solvent winterization. Each solvent was cooled to –20°C and then combined with sample 3 (heavily sedimenting oil) and sample 28 (oil recovered from the filter cake) to determine which solvent was most effective at producing sediment.

Samples 3, 5, 9, 13, 16, 17, and 29 were combined with cold solvent at low (30%) and high (70%) concentrations. Acetone was also used at 10%, as used in previous studies (10,3). Samples 3, 7, 9, 16, and 17 were combined with cold acetone concentrations of 10–90% solvent to determine the most effective solvent/oil combination. Samples 1–6, 9–14, 18, 19, 21–24, and 29 were then screened with acetone (30 and 70%).

Comparison of cold tests. The ability of the various tests to determine the cloud potential of samples (3–7, 9–14, 16–19, and 21–24) were compared using the AOCS cold test, a commercial refiner's test, cold storage at 1–3 and 7°C, 10% acetone, and 30 and 70% acetone, as developed in this study.

RESULTS AND DISCUSSION

Cold tests. Of the 25 samples tested for clouding potential using the AOCS cold test, all but one sample (sample 12) passed. The oils were kept at 0°C and observed again after 24 h, by which time two additional oils (samples 6, 7) had begun to cloud. Each of the three oils that failed the AOCS cold test was known to sediment at room temperature; however, not all room temperature sedimenting oils were detected, and no other oil was detected. Cold-pressed oil (sample 20) was also tested, and while the oil passed the cold test to at least 22 h, a sample of the same oil cooled to 7°C clouded by 5.5 h.

The samples were then evaluated using the commercial refiner's test. Samples 2 and 4, which both sedimented at room temperature when obtained, failed the test within 48 h, but no other oils failed after four additional days. Oils that pass the test are considered not needing winterizing, but this cold test failed to detect oils known to sediment at room temperature. Sample 12, which failed the AOCS test, passed the commercial refiner's test.

Our previous study (2) has shown that lower temperatures result in more rapid sediment formation. To investigate whether reducing the temperature of the commercial refiner's test may lead to more accurate results, oils were stored at 7°C for 7 d. At 24 h, while eight samples had passed (samples 14, 16–19, and 21–24), the remaining samples all failed. After 7 d, all oils except four refined canola oils (samples 14, 16, 17, and 19) developed cloudiness.

The oils were also stored at 1–3°C for 7 d. All oils sedimented, most oils clouding more rapidly than they had at the higher temperature. While these lower temperature cold tests are able to produce cloudiness in canola oils, they are lengthy and do not discriminate between those oils which cloud only while refrigerated and those which sediment at room temperature.

Solvent-aided cold tests. Alternative cold tests have been proposed to provide more accurate and rapid sediment prediction (3, 8, and 10). Hexane, acetone, petroleum ether, and acetone/hexane were combined with samples 3 and 28 to determine which solvent was the most effective at producing sediment (Table 1). The filter cake extract (sample 28) immediately clouded when shaken with each of the solvents. Clouding was greater with the acetone/hexane combination and with acetone alone than with petroleum ether or hexane.

TABLE 1
Solvent Precipitation of Sedimenting Canola Oils (samples) and the Chloroform Extract of Canola Filter Cake (sample 28)[a]

		Filter cake extract		Sedimenting canola oil	
Method	Oil concentration	0 h 7°C	18 h –20°C	18 h 7°C	18 h –20°C
Acetone/hexane (85:15, vol/vol)	10% oil	5	5	1	5
	50% oil	3	5	1	5
Cold acetone	10% oil	5	5	3	3
	50% oil	4	5	4	2
Cold petroleum ether	10% oil	2	2	0	0
	50% oil	2	2	0	0
Cold hexane	10% oil	2	2	0	0
	50% oil	3	4	0	0

[a]Scoring scale: 0 = clear, 1 = few, small crystals, 2 = more, small crystals, 3 = more, larger crystals, 4 = majority of the oil clouded by large crystals, 5 = maximum clouding. Both samples were clear after 24 h at room temperature with any solvent combination.

TABLE 2
Cold-Test Results After 18 h at 0°C[a]

Sample	30% a/h	70% a/h	40% Acetone	70% Acetone	10% Acetone	30% Hexane	70% Hexane	30% Pet. ether	70% Pet. ether	Control (100% oil)
3—Sediments at room temperature	5	3	5	3	5	0	0	0	0	0
5—Sediments at room temperature	5	1	5	3	5	0	0	0	0	0
9—Sediments heavily at 7°C	0	0	0	3	5	0	0	0	0	0
13—Sediments at at 7°C, had sedimented at room temperature but much of this removed for analysis	1	0	1	2	5	0	0	0	0	0
16—Sediments only lightly at 7°C	0	1	0	3	0	0	0	0	0	0
17—Sediments only lightly at 7°C	0	0	0	1	0	0	0	0	0	0
29—Sunflower oil, sediments at room temperature	4	2	5	2	1	0	0	0	0	0

[a]Oil samples were combined with solvents in the ratios described. a/h indicates combined acetone/hexane (85:15). Samples described as sedimenting at 7°C do not sediment at room temperature unless indicated. Scoring: 0 = clear, 1 = tiny crystals, 2 = small crystals, 3 = large crystals, 4 = large crystals, beginning to settle, 5 = maximum crystal formation and settling. All oils are canola except sample 29 (sunflower oil).

Canola oil, sample 3, did not cloud immediately with any solvent, or with hexane or petroleum ether during storage. Both the canola oil (sample 3) and the filter cake extract (sample 28) remained clear with all of the solvents after 24 h at room temperature, the crystals in the filter cake samples redissolving during storage. The most appropriate conditions for cold tests were with acetone/hexane or acetone alone, and cold storage.

These results were confirmed using a wider range of sedimenting oils including the canola and sunflower oils which sediment at room temperature, and canola oil, which only sediments when refrigerated (Table 2). As found previously, acetone was the most effective solvent; acetone/hexane also produced sedimentation. While none of the control samples sedimented within 18 h (Table 2), the addition of acetone/hexane or acetone allowed more rapid formation of crystals. Variations on standard cold tests generally allow more time for cloudiness to develop. The use of solvents to decrease testing time allows more efficient oil processing, as the oil does not have to be held in storage as long.

Acetone was chosen to screen the oils because it was previously shown to be the most effective in producing sediment. Acetone was combined with each of the oils previously tested to determine which ratio of acetone to oil would best indicate problem oils. Acetone at 40% or less discriminated between oils (Table 3) that sedimented at room temperature and those that did not. Solvent/oil combinations with 10 to 40% acetone performed similarly, as did combinations with 60 to 90% acetone. While lower proportions of acetone produced greater amounts of sediment for samples 3 and 7, both of which sediment at room temperature, higher proportions of acetone produced sediment in more of the oils.

Proportions of 30 and 70% solvent were then chosen as representative of each group. To test the efficiency of the acetone cold test in screening for oils which sediment, 19 oils were combined with 30 or 70% cold acetone, then stored in an ice-water bath and observed for cloudiness development. Samples with 70% acetone precipitated more heavily than did samples with 30% acetone (Table 4). Oils that did not sediment at either room temperature or when refrigerated remained clear for the time tested at both concentrations of acetone. Oils were observed after 3, 6, and 24 h. During this time, oils that had not previously sedimented under any conditions passed the test. Of the oils which sedimented at room temperature, all failed with 30% acetone after 24 h, and with 70% acetone after 6 h. These results show that the 70% acetone test is more rapid, and therefore more suitable for industrial use. All oils, which sediment while refrigerated but not at room temperature, also failed within 6 h with 70% acetone, except for sample 13. This sample sedimented only very lightly in the refrigerator after long term-storage, but was not sediment-free. The addition of acetone was not effective in detecting cloudy cold-pressed oils at less than 24 h. Cold-pressed oil samples 22 and 23 remained clear during the test, while samples 21 and 24 clouded only at 24 h.

TABLE 3
Acetone Cold-Test After 18 h at 0°C[a]

Sample	Acetone concentration (%)								
	10	20	30	40	50	60	70	80	90
3—Sediments at room temperature	5	5	5	5	3	4	3	3	3
7—Sediments at room temperature	5	5	5	5	4	4	4	3	3
9—Heavily sediments at 7°C, not at room temperature	0	0	0	0	0	4	4	4	3
16—Sediments only lightly at 7°C	0	0	0	0	1	3	1	4	1
17—Sediments only lightly at 7°C	0	0	0	1	0	0	0	0	0

[a]Percentages are percentage solvent in oil. Acetone at 40% or lower discriminates between oils which sediment at room temperature and those that do not, while higher percentages of acetone are less selective. Scoring scale is as for Table 2.

TABLE 4
Development of Oil Combined with Cold Acetone and Stored in an Ice-Water Bath[a]

Sample	30% Acetone 3 h	30% Acetone 6 h	30% Acetone 24 h	70% Acetone 3 h	70% Acetone 6 h	70% Acetone 24 h
Oil sedimenting at room temperature						
1	1	1	3	1	4	4
2	0	0	1	0	2	3
3	2	4	4	3	4	4
4	3	4	4	1	4	4
5	1	3	4	1	4	4
6	1	3	4	1	4	4
29, Sunflower oil	0	1	4	2	3	4
Oils sedimenting only at 7°C						
9, Heavy sediment	0	3	3	0	3	4
10, Heavy sediment	0	0	0	1	2	3
11	0	1	4	0	3	4
12	0	3	4	0	4	4
13, Small flakes	0	0	0	0	0	*
14, Small flakes	0	0	1	2	3	4
Oils not sedimenting at room temperature or at 7°C						
18, Sediment removed	0	0	0	0	0	0
19	0	0	0	0	0	0
Cold-pressed oils						
21	0	0	0	0	0	1
22	0	0	0	0	0	0
23	0	0	0	0	0	0
24, Small flakes at 7°C	0	0	1	0	0	3

[a]Scale: 0 = clear, 1 = hazy, 2 = small crystals, 3 = larger or more crystals, 4 = setting crystals. * = one large flake in clear oil. Oils are canola unless specified.

TABLE 5
Comparison of Cold-Test Methods[a]

Sample	AOCS cold test	Commerical refiner's test	Cold storage, 7°C, 24 h	Cold storage 1–3°C	10% Acetone 6 h	30% Acetone 6 h	70% Acetone 6 h
Oil sedimenting at room temperature							
3	Pass	Pass	Fail	Fail	Fail	Fail	Fail
4	Fail	Fail	Fail	Fail	Fail	Fail	Fail
5	Pass	Pass	Fail	Fail	Fail	Fail	Fail
6	Pass (fail at 24 h)	Pass	Fail	Fail	Fail	Fail	Fail
7	Pass (fail at 24 h)	Pass	Fail	Fail	Fail	Fail	Fail
Oils sedimenting only at 7°C							
9	Pass	Pass	Fail	Fail	Pass	Fail	Fail
10	Pass	Pass	Fail	Fail	Pass	Pass	Fail
11	Pass	Pass	Fail	Fail	Fail	Fail	Fail
12	Fail	Pass	Fail	Fail	Fail	Fail	Fail
13	Pass	Pass	Fail	Fail	Pass	Pass	Fail
14	Pass	Pass	Pass	Fail	Fail	Pass	Fail
16	Pass	Pass	Pass	Pass	Pass	Pass	Fail
17	Pass	Pass	Pass	Pass	Pass	Pass	Fail
Oils not sedimenting at room temperature or at 7°C							
18	Pass	Pass	Pass	Pass	Pass	Pass	Pass
19	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Cold-pressed oils							
21	Pass	Pass	Pass	Fail	Fail	Pass	Pass
22	Pass	Pass	Pass	Fail	Pass	Pass	Pass
23	Pass	Pass	Pass	Fail	Pass	Pass	Pass
24	Pass	Pass	Pass	Fail	Pass	Pass	Pass

[a]Oils that were clear at the observation time indicated passed the cold test, whereas oils that had begun to cloud fail.

Comparison of cold tests. Each of the cold tests was repeated, comparing selected oils of known sedimenting behavior (Table 5). Of all the samples tested, only sample 4 failed all seven tests. Samples 3, 5, 6, and 7, which all sediment at room temperature, passed the AOCS and commercial refiner's test. These oils would have been allowed to proceed unwinterized based on those tests. The use of any of the five remaining tests would have detected these oils as potentially cloudy oil and in need of further treatment.

Both lower cooling temperatures and solvent cold tests were more effective than the AOCS and commercial refiner's tests at detecting oils which sediment when cooled to 7°C. Acetone at 70% identified each oil as a potential cloudy oil. Canola oil is used as a salad oil and must therefore remain clear when refrigerated. The use of acetone at 70% as a cold test may allow more rapid identification of oils which will go cloudy when refrigerated. The oils, which do not sediment either at room temperature or at 7°C, remained clear for each of the tests, although cloudiness developed in both oils after 7 d at 1–3°C. The cold-pressed oils failed only storage at 1–3°C and in 10% acetone. Tests for cold-pressed oils require further studies.

ACKNOWLEDGMENTS

NSW Agriculture and the Australian Oilseed Federation funded this work. The advice and assistance of Ron Bowrey, Peter Cox, Rod Smith, and Geoff Black are acknowledged.

REFERENCES

1. Gao, Z., and R.G. Ackman, Gradient Density Isolation and Fractionation of Haze-Causing Solids of Canola Oil, *J. Agric. Food Chem. 68*:421–430 (1995).
2. Botha, I., R.J. Mailer, and K. Robards, Evaluation of the Role of Saturated Fatty Acids in Sedimenting Canola Oils, *J. Am. Oil Chem. Soc. 77*:429–435 (2000).
3. Liu, H., R. Przybylski, and N.A.M. Eskin, Turbidimetric Measurement of Haze in Canola Oil by Acetone Precipitation, *Ibid. 73*:1557–1560 (1996).
4. Turkulov, J., E. Dimic, D.J. Karlovic, and V. Vuksa, The Effect of Temperature and Wax Content on the Appearance of Turbidity in Sunflower Seed Oil, *Ibid. 63*:1360–1363 (1986).
5. Sulaiman, M.Z., N.M. Sulaiman, and S. Kanagaratnam, Triacylglycerols Responsible for the Onset of Nucleation During Clouding of Palm Olein, *Ibid. 74*:1553–1558 (1997).
6. Chulu, C.L., P. Barlow, and M. Hole, Cloudiness in Zambian Sunflower Oil: Effect of Storage Temperature and Wax Levels, *Trop. Sci. 29*:33–38 (1989).
7. Brimberg, U.I., and I.C. Wretensjo, Rapid Method for Determination of Wax in Sunflowerseed Oils, *Ibid. 56*:857–860 (1979).
8. Leibovitz, Z., and C. Ruckenstein, Winterization of Sunflower Oil, *Ibid. 61*:870–872 (1984).
9. Baltanas, M.A., H. Molina, and C. Silva, Rapid Method for Predicting the Appearance of Turbidity in Sunflower Oil and Their Comparison with Cold Tests, *Ibid. 75*:363–370 (1998).
10. Mertens, W.G., L.J. Rubin, and B.F. Teasdale, The Production of Salad Oil by Fractional Crystallization with Solvents, *Ibid. 38*:286–289 (1961).

[Received June 28, 2000; accepted January 4, 2001]