# Turbidimetric Measurement of Haze in Canola Oil by Acetone Precipitation

**Hua Liu[a,*], Roman Przybylski[b], and N.A. Michael Eskin[b]**

Departments of [a]Food Science and [b]Foods and Nutrition,
University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

**ABSTRACT:** Formation of turbidity in canola oil was facilitated with addition of acetone, and a method to measure the sediment content based on the oil turbidity has been developed. Canola oil was mixed with acetone at the ratio of 60:40, and the turbid solution developed in an ice bath for 20 min. The turbidity of the oil solution was determined by a turbidimeter. The relation between turbidity of the oil solution and sediment content was nonlinear and could be correlated by a second-order polynomial. There was no difficulty in the development of turbidity in canola oil solutions in the presence of added lecithin (2%, w/w). However, with added lecithin, turbidity was 23% higher at the same sediment content.
*JAOCS 73*, 1557–1560 (1996).

**KEY WORDS:** Acetone–canola oil solution, lecithin effect, sediment content, turbidity measurement.

Development of haze, or sediment, is a common problem in some vegetable oils because of the solidification of high-melting constituents present in the oils (1,2). This phenomenon also has been reported to occur in canola oil. The major haze causing constituents in canola oil are waxes (3–5), being in the range of 18 to 73 ppm (4,5). For sunflower oil, a much higher concentration, 200–4700 ppm, has been found (2,6). Accurate determination of the sediment content in oils, however, is difficult. This information is necessary for efficient processing of oils particularly with canola oil because of the sporadic nature of the problem. One common test, referred to as the cold test, is a lengthy procedure and only gives qualitative information about sediment (7). Canola oil which has been winterized and passed the cold test may also form haze occasionally (Dyck, J., personal communication). The gas-liquid chromatographic (GLC) determination of wax content in sunflower seed oil developed by Morrison and Robertson (8), is quantitative but not a quick method.

A nephelometric method has been developed by Brimberg and Wrentensjo (7) to relate the turbidity of cold sunflower oil to the sediment content. This is a quick method provided that the turbidity of oil can be successfully developed in a timely fashion. When the sediment content is low, however, it may require many days for turbidity to be developed in the cold oil (9). Under normal storage conditions, these oils, for kinetic reasons, may remain clear for a period of time but will eventually develop a sediment upon prolonged storage. Other factors like the presence of natural crystal inhibitors (such as phospholipids) are also known to affect sediment formation and therefore influence the accuracy of the measurement (3).

The turbidity method was modified by Morrison (6) by using organic solvents such as acetone or a mixture of hexane/acetone to expedite the turbidity development of sunflower oil. A solvent content of 50% (wt/vol) or higher was recommended (6,10). This method was also found to be applicable to crude sunflower oil, where the turbidity measurement without solvent was difficult due to the presence of phospholipids in the oil. Phospholipids are known to retard crystallization of sediment in oil by inhibiting the crystal growth process (3). The content of phospholipids in crude oils varies from oil to oil (11). In crude canola oil, a phospholipid content about 2–3.6% has been reported (12).

The effects of solvent on the phase transition behavior of sediment and the solution viscosity in canola oil–solvent systems have recently been investigated (13, 14). These studies revealed that addition of a solvent such as acetone substantially reduced the solution viscosity (by a factor of 459 at 0°C), thereby facilitating the crystallization process of sediment in the oil solution. A level of 30–40% solvent was found to be sufficient to bring about the desired effects in canola oil (14).

Comparison between canola and sunflower oil sediments revealed significant differences in both the chemical and physical properties (15) of the sediments. Canola sediment contained a lower amount of wax esters, but the fatty acids in the esters had longer carbon chains. Variation in the sediment composition will influence the phase transition behavior (15) and consequently the relation between turbidity and sediment content. Further studies on the turbidity of canola oil at optimum solvent levels are necessary. This study aims to establish the relation between turbidity and sediment content and the conditions for measurement in canola oil. The effect of phospholipids on the development of turbidity in a canola oil solution was also investigated to determine the applicability of the technique to crude canola oils.

*To whom correspondence should be addressed.

## MATERIALS AND METHODS

Refined, bleached, and deodorized canola oil was obtained from a Western Canadian oil processor. The oil was stored at 0°C for a week, and then filtered using a Buchner funnel with a filter paper topped with a layer of diatomaceous earth (Sigma, St. Louis, MO) under vacuum. The filtered canola oil was considered sediment-free and used throughout the study.

Canola oil sediment was separated from filter cakes obtained during winterization of canola oil. The detailed procedure for sediment separation was described previously (9,16). Briefly, this involved extraction of filtration cakes with hot chloroform, stripping off the solvent by vacuum distillation, precipitating the sediment at low temperature, and separation of the sediment by centrifugation from the solution. The sediment obtained was washed twice with cold petroleum ether (2°C) to remove any residual oil from the material (16).

The melting properties of the canola sediment were determined using a Dupont thermal analyzer (9900; Wilmington, DE) equipped with a Dupont 910 DSC cell. A single melting endotherm at around 75°C was observed for the material in agreement with previous studies (9).

An appropriate amount of sediment was dissolved in canola oil to obtain a stock solution (1000 ppm sediment). Oil samples with various amounts of sediment were prepared by dilution of the stock solution with canola oil. In turbidity analysis, canola oil samples were first heated to about 130°C to melt the sediment and cooled to about 40°C. Acetone (analytical grade, Sigma) was then mixed with the oil at a level of 40% (w/w) solvent in cuvettes (ø27; HF Scientific, Inc., Fort Myers, FL). The turbidity was developed by placing the cuvettes in an ice bath for fixed periods of time. The cuvettes were removed from the ice bath and wiped dry with tissue wipers before inserted into the measuring chamber of a turbidimeter. A Fisher model DRT 100 turbidimeter (H.F. Instruments Ltd., Ontario, Canada) was used for the turbidity measurement. The instrument was calibrated against a reference standard supplied by the manufacturer. Preliminary experiments showed that moisture condensation on the cold walls of a cuvette could elicit erroneous results. This was overcome by placing the instrument and taking the turbidity measurements in a cold storage room (5°C).

To investigate the effect of phospholipids on the turbidity of sediment in canola oil, lecithin (L-α-Lecithin from soybean; Calbiochem, San Diego, CA) was added in canola oil at a level of 2% (w/w), and the samples were subjected to the same treatments before turbidity measurements.

## RESULTS AND DISCUSSION

The turbidity of the oil solution should be fully developed in order for the turbidity method to accurately measure the sediment content in canola oil. Figures 1 and 2 show the typical curves for turbidity development of canola oil solutions as a function of cooling time at two representative sediment concentrations. The turbidity of the oil solutions increased rapidly during the first few minutes and thereafter slowly, reaching a plateau

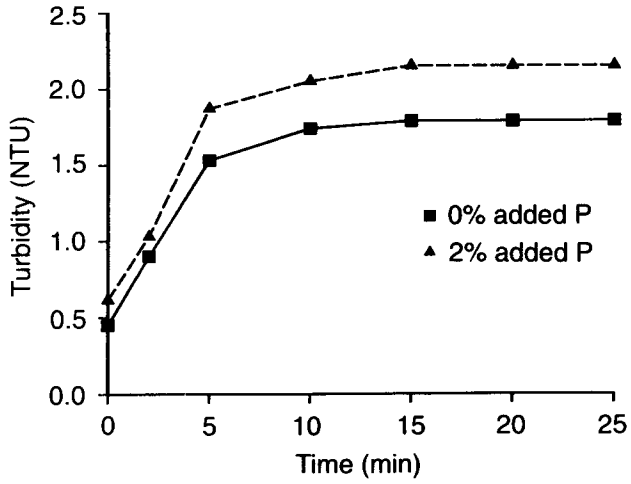


**FIG. 1.** Turbidity of canola oil containing 50 ppm sediment as a function of cooling time at 0°C. The turbidity for corresponding oils with 2% lecithin was also shown in broken line.

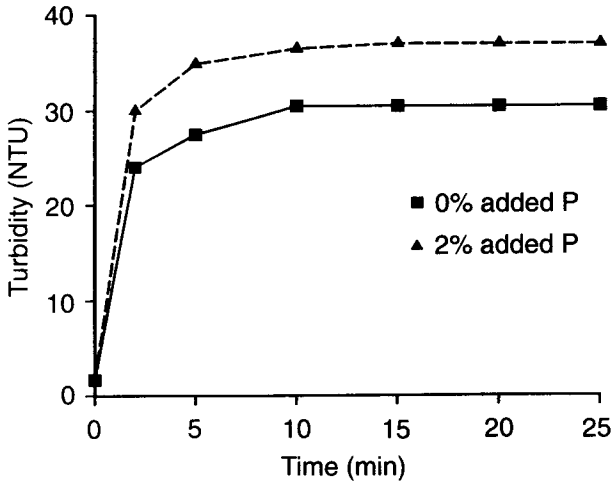


**FIG. 2.** Turbidity of canola oil containing 1000 ppm sediment as a function of cooling time at 0°C. The turbidity for corresponding oils with 2% lecithin was also shown in broken line.

value at about 10 to 15 min. Turbidity which developed after about 20 min was stable and uniform. This was found for all the oil samples tested over a sediment concentration of 0–1000 ppm and prolonged treatment in an ice bath for up to an hour did not affect the turbidity readings. The same results were obtained with canola oils containing 2% of added lecithin with the corresponding turbidity development curves shown in Figures 1 and 2, respectively. Based on these findings, 20 min of development time was chosen for the turbidity analysis of canola oil.

Figure 3 shows the turbidity (NTU) of canola oil solution as a function of sediment content (ppm). The canola oil solution without added sediment gave 0.67 in turbidity which was attributed to the coloring material present in the oil. It is evident from Figure 3 that the relation between turbidity and the sediment content of canola oil was nonlinear. This

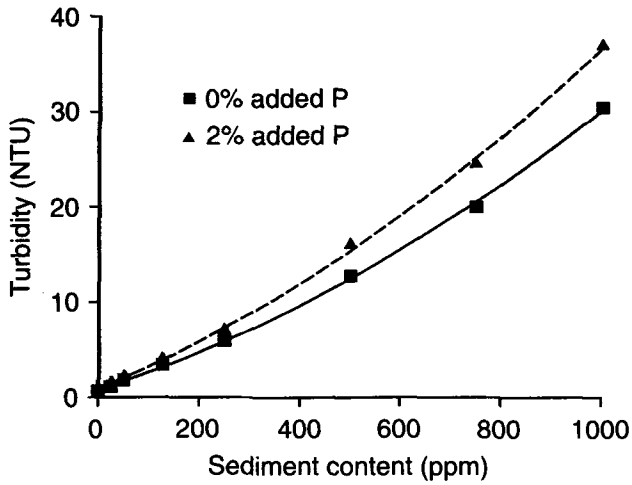


**FIG. 3.** The correlation between turbidity (NTU) and sediment content (ppm) in canola oil at 40% (wt) acetone with or without 2% lecithin.

was in agreement with previous studies on turbidimetric measurement of sunflower oil (6,7). However, Moulton (10) found a linear relation between the turbidity and wax content in sunflower oil (10). Theoretically, the turbidity of a solution is a complex function of concentration and particle size (17). Accordingly, a nonlinear correlation between the turbidity and sediment concentration is expected. A quadratic equation was found to describe the experimental data in the present study, i.e.,

$$\tau = 0.84 + 1.694 \times 10^{-2}\, w + 1.249 \times 10^{-5}\, w^2 \quad [1]$$

or

$$w = 48.58\tau - 0.484\tau^2 - 31 \quad [2]$$

where $\tau$ is the turbidity (NTU), and $w$ the sediment content (ppm) in canola oil, respectively. A correlation coefficient higher than 0.999 was obtained. The coefficient of variation for turbidity measurement was 5% by average.

A polynomial equation similar to Equation 1 was also developed by Morrison (6) for correlating the turbidity in sunflower oil with wax content. However, there appears to be several errors in the equation. Closer examination of the equation indicated that NTU = $1.68 \times 0.056$ ppm + $1.3 \times 10^{-5}$ ppm$^2$ could explain the data presented (the original equation was $y = 1.68 \times 0.56\,(x) + 1.3 \times 10^{-5} \times 2$, where $y$ = ppm wax, $x$ = turbidity in NTU). Compared to sunflower oil, the turbidity of canola oil found in the present study was lower at the same sediment content. This was probably due to the variation of sediment composition in these oils; sunflower sediment contains higher proportion of wax esters than canola (15).

In a study on turbidimetric measurement of wax content in sunflower oil without solvent, Brimberg and Wrentensjo (7) reported that the technique was not applicable to crude oils because phospholipids present inhibited turbidity development. When acetone was used in turbidity measurement, however, turbidity development in canola oil samples that contained a significant amount of lecithin was successful as shown in Figures 1 and 2.

Figure 3 shows that the turbidity measured by the solvent crystallization technique was higher in the presence of 2% added lecithin. The variation in turbidity was greater as the sediment content in canola oil increased. This deviation did not appear to be due to the lecithin precipitation since canola oil containing 2% lecithin with no added sediment gave turbidity readings similar to the blank oil sample. Phospholipids could be precipitated by acetone as reported by Sinram (18) who employed 50 mL acetone per 0.33 g oil. The solvent level used in the present studies was, however, much lower; it appeared that a higher solvent/oil ratio was required for the phospholipids to crystallize out of the solution. Previous studies showed that phospholipids retarded sediment crystal growth (3) so that smaller crystal particles could be a factor responsible for the higher turbidity readings in these samples. The relative change in turbidity in the presence of phospholipid (defined as the absolute difference in turbidity divided by the turbidity for oil without added lecithin) was about 23% by average over the sediment contents studied. This suggested that when the turbidity technique is applied to crude canola oil, the sediment content calculated from the normal calibration curve would be higher. The correlation between the turbidity and sediment content for canola oil in the presence of 2% lecithin could also be described by a polynomial equation, i.e.,

$$\tau = 0.94 + 2.184 \times 10^{-2}\, w + 1.399 \times 10^{-5}\, w^2 \quad [3]$$

or

$$w = 38.85\tau - 0.299\tau^2 - 28 \quad [4]$$

where the symbols are as defined above.

## ACKNOWLEDGMENT

The authors acknowledge the financial support from the Natural Science and Engineering Research Council of Canada.

## REFERENCES

1. Weiss, T.J., Salad Oil Manufacture and Control, *J. Am. Oil Chem. Soc. 44*:146A–148A (1967).
2. Leibovitz, Z., and C. Ruckenstein, Winterization of Sunflower Oil, *Ibid. 61*:870–872 (1984).
3. Liu, H., C.G. Biliaderis, R. Przybylski, and N.A.M. Eskin, Effects of Crystallization Conditions on Sedimentation of Canola Oil, *Ibid. 71*:409–415 (1994).
4. Hu, X., J.K. Daun, and R. Scarth, Characterization of Wax Sediment in Refined Canola Oils, *Ibid. 70*:535–537 (1993).
5. Gao, Z., and R.G. Ackman, Gradient Density Isolation and Fractionation of Haze-Causing Solids of Canola Oil, *J. Sci. Food Agric. 68*:421–430 (1995).
6. Morrison, III, W.H., Rapid Determination of Wax in Sunflower Seed Oil, *J. Am. Oil Chem. Soc. 59*:284–285 (1982).
7. Brimberg, U.I., and I.C. Wrentensjo, Rapid Method for Determination of Wax in Sunflowerseed Oils, *Ibid. 56*:857–860 (1979).
8. Morrison, III, W.H., and J.A. Robertson, Solvent Winterization of Sunflower Seed Oil, *J. Am. Oil Chem. Soc. 52*:148–150 (1975).

9. Liu, H., C.G. Biliaderis, R. Przybylski, and N.A.M. Eskin, Phase Transitions of Canola Oil Sediment, *Ibid. 70*:441–448 (1993).
10. Moulton, K.J., Turbidimetric Measurement of Wax in Sunflower Oil, *Ibid. 65*:367–368 (1988).
11. Haraldsson, G., Degumming, Dewaxing and Refining, *Ibid. 60*:251–256 (1983).
12. Sosulski, F., R. Zadernowski, and K. Babuchowski, Composition of Polar Lipids in Rapeseed, *Ibid. 58*:561–564 (1981).
13. Liu, H., C.G. Biliaderis, R. Przybylski, and N.A.M. Eskin, Solvent Effects on Phase Transition Behaviour of Canola Oil Sediment, *Ibid. 72*:603–608 (1995).
14. Liu, H., R. Przybylski, and N.A.M. Eskin, Influence of Solvent Content on Phase Transition Temperature of Oil Sediment and Solution Viscosity in Acetone–Canola Oil Systems, *Ibid. 73*:1137–1141 (1996).
15. Liu, H., R. Przybylski, K. Dawson, N.A.M. Eskin, and C.G. Biliaderis, Comparison of the Composition and Properties of Canola and Sunflower Oil Sediments with Canola Seed Hull Lipids, *Ibid. 73*:493–498 (1996).
16. Przybylski, R., C.G. Biliaderis, and N.A.M. Eskin, Formation and Partial Characterization of Canola Oil Sediment, *Ibid. 70*:1009–1015 (1993).
17. Reddy, S.R., and H.S. Fogler, Emulsion Stability: Determination from Turbidity, *J. of Colloid and Interface Sci. 79*:101–104.
18. Sinram, R.D., Nephelometric Determination of Phosphorus in Soybean and Corn-Oil Processing, *J. Am. Oil Chem. Soc. 63*:667–670 (1986)

[Received December 12, 1995; accepted July 17, 1996]