

Solvent Effects on Phase Transition Behavior of Canola Oil Sediment

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ABSTRACT: Differential scanning calorimetry (DSC) was used to study the melting and crystallization behavior of waxy sediment in canola oil and in mixtures (1:1, w/w) of oil and acetone or hexane under dynamic heating/cooling regimes. In the presence of a solvent, the DSC melting peak of sediment shifted to lower temperatures, suggesting that sediment was more soluble in the solvent/oil systems than in oil alone. This effect was greater with hexane than with acetone. The influence of a solvent on crystallization was more complex. With inclusion of hexane, the crystallization temperature of sediment was always lower than that in oil. With acetone, however, the crystallization temperature of sediment was slightly lower at high sediment content, but higher at low sediment content than in oil alone. The differences in melting and crystallization behavior of sediment in canola oil and the solvent/oil systems were attributed to solubility and viscosity effects. Variation in the crystalline solid structures of sediment was not evident from the melting enthalpies associated with the phase transformation. *JAACS* 72, 603–608 (1995).

KEY WORDS: Calorimetry, canola oil sediment, melting and crystallization temperatures, solvent crystallization.

Over the past few years, several studies have examined the chemical composition and physical properties of the minor lipid constituents that cause haze in canola oil (1–4). In these studies, canola oil sediment was isolated either by a combination of filtration and centrifugation (1,2) or by a gradient density technique (3), and the composition was analyzed chromatographically (2–4). Wax esters of long-chain fatty acids and long-chain fatty alcohols with carbon chainlengths of 15–32 were identified as the major constituents of the sediment. Although these techniques give valuable information about the composition of sediment, the analysis is tedious and does not lend itself to a rapid estimate of the amount of sediment in oil. The sediment content of canola oil may vary with variety and growing area, as well as seasonal and environmental conditions, and may require different processing conditions for its removal from the oil. Rapid estimation of the sediment content in oils is therefore important if the quality

and stability of the products are to be maintained. This can further ensure proper processing so that hazy appearance in processed canola oils can be avoided.

Several methods have been developed over the past few years for measuring the wax content in sunflower seed oil (5–7). These include gravimetric analysis, gas–liquid chromatography (GLC) analysis of the alcohols released upon hydrolysis of the wax esters, and a laser-based technique to detect wax microcrystallites. All these methods require specialized equipment, prolonged extraction, and chemical treatments. Another technique, based on the solvent winterization principle (8), was developed by Morrison (5). According to this method, acetone, or an acetone/hexane mixture is added to the oil to bring about crystallization of wax, and the waxy sediment content is measured by a turbidimeter. This technique is rapid and requires less expensive equipment. Application of the method has been reported even for crude sunflower oils where the presence of phospholipids may cause problems, due to retardation of wax crystallization (5).

In solvent crystallization, addition of a solvent to oil is assumed to make waxy substances less soluble in the solvent/oil mixture, resulting in a nearly complete removal of waxes by crystallization (5). However, little is known about the phase relations of wax or sediment constituents in a solvent/oil mixture. In a series of investigations on solvent winterization of sunflower and cottonseed oils, Skau and co-workers (9–11) studied the amounts of solids removed from oils at various chilling temperatures and ratios of solvent/oil. According to these workers, for certain oil/acetone ratios at a temperature about 5°C below that required for adequate winterization, two liquid phases could be obtained. This liquid separation could be suppressed if a mixture of hexane and acetone was used. Addition of a second solvent seems to affect the phase relations of crystallizable constituents in the oil solution. Thus, knowledge of the effect of individual solvents on the phase transition behavior of waxy substances in oil will be needed to completely understand such multicomponent systems.

Differential scanning calorimetry (DSC) has been used extensively over the past years to study protein denaturation, water properties in foods, as well as phase transitions of food carbohydrates, both sugars and polysaccharides (12). DSC has also proven to be particularly useful in studying polymor-

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phic transformation and crystallization kinetics of fats (13). DSC, as compared to other techniques, is rapid and provides both qualitative and quantitative information of heat-induced transitions, such as melting and crystallization. The specimens can be conveniently sealed during DSC analysis, thus preventing the loss of volatile components from the sample.

In the present report, the DSC was employed to study the phase transition behavior of canola oil sediment in mixtures of a solvent (acetone or hexane) and canola oil. Experiments were conducted over a range of sediment contents to probe the solubility and crystallization behavior of sediment in the presence of a solvent. This approach may provide insight into the mechanism of solvent crystallization (winterization) of high-melting oil constituents. Moreover, the DSC data could lead to a better selection of a solvent or solvent mixture in developing an accurate turbidimetric test for determining the waxy sediment content in canola oil.

MATERIALS AND METHODS

Canola filter cakes, collected by filtering winterized (6–10°C) canola oil, were obtained from a Western Canadian oil processor. The filter cakes were extracted with hot chloroform, and the extract was concentrated by vacuum distillation in a rotary evaporator (40°C). The concentrated solution was stored in a refrigerator for a week at around 0°C, and the precipitate was separated from the solution by centrifugation (16,300 × g). The sediment was washed twice with cold petroleum ether (2°C) to remove any residual oil from the material (4).

Refined, bleached, and deodorized canola oil was kept at 0°C for a week and then filtered through filter paper (Whatman No. 541; Maidstone, England) under vacuum with a filter aid (diatomaceous earth; Sigma, St. Louis, MO) to remove any solids formed. The filtered canola oil was used throughout the study.

Solvent/oil solutions were prepared by mixing acetone or hexane with canola oil in the proportion of 50:50 by weight. Such a solvent/oil ratio was employed in earlier investigations on solvent crystallization of waxes and winterization of oils (5,9–11). Phase separation was not observed in the solutions for the oil/solvent ratio used.

All the solvents used were of analytical grade and were purchased from Sigma Chemical Co.

DSC measurements were made with a thermal analyzer (Dupont 9900; Dupont, Wilmington, DE) equipped with a Dupont 910 DSC cell. The DSC samples were prepared by weighing a specified amount of canola sediment into the DSC crucibles, adding the desired amount of oil or the solvent/oil solution into the samples with a microsyringe, and hermetically sealing the crucibles. When the sediment content was low, the solutions were prepared by mixing the specified amounts of sediment and oil or oil/solvent solutions in capped vials. The solid sediment was evenly dispersed into the solution by heating. A desired amount (5–7 mg) of the mixture was then transferred to the DSC crucibles and hermetically

sealed. Samples encapsulated in DSC crucibles were scanned in the calorimeter over a typical temperature range of 5–80°C, or higher for some samples where phase transitions occurred at high temperatures. An empty DSC crucible was used as an inert reference. In a typical DSC run, a sample was heated at 10°C/min to the upper end temperature, held isothermally for 1 min and thereafter cooled at 10°C/min to the lower end temperature. The programmed cooling rate was achieved by using a cooling accessory with icy water as a coolant. The presence of oil and sediment greatly suppressed the evaporation of the solvent, and the loss of the solvent during a DSC experiment was minimal, as evident from the smooth DSC base line. Should there be any evaporative loss of solvent from the samples, a noisy baseline, or even a peak, should have been observed. The DSC instrument was calibrated with indium before use (1). All DSC traces were normalized based on a constant weight of sediment solids.

RESULTS AND DISCUSSION

Figure 1 shows typical DSC thermal curves for sediment in canola oil during the heating/cooling cycle. When the sample was heated, an endothermic peak was observed, indicating the melting of the solid sediment constituents. Upon subsequent cooling to a sufficiently low temperature, a DSC exotherm was obtained. This is attributed to crystallization of sediment constituents from the oil. The shifting of the crystallization exotherm to a lower temperature is due to the supercooling effect (1). The crystallization peak was also sharper and occurred in a narrower temperature range, as compared with the broader melting endotherm. When the sample was supercooled to the temperature corresponding to the critical supersaturation, spontaneous nucleation occurred (14), resulting in a narrow and sharp crystallization peak. The area under the DSC first-order transition corresponds to the enthalpy for the phase transformation. Despite the differences in the DSC peak shapes and temperature locations, the en-

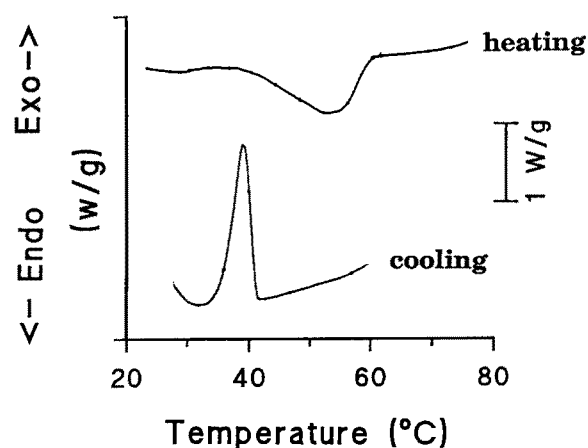


FIG. 1. Differential scanning calorimetry thermograms of sediment in canola oil at 1% (w/w) sediment content, obtained during a heating and cooling cycle, showing a melting endotherm (endo) and a crystallization exotherm (exo).

thalpy for crystallization was the same as that for melting. This may indicate that the same amount of lipid constituents crystallized as that melted during the heating cycle.

Figure 2 shows the melting and crystallization thermal curves of sediment in a mixture of canola oil and acetone. As in canola oil alone, an endothermic peak occurred during heating corresponding to the melting transition of sediment, whereas exothermic (crystallization) peaks were observed during cooling. The cooling profile in Figure 2 also shows a distinct small exothermic peak after the major transition, suggesting that some lipid species exhibit delayed crystallization from the liquid phase. This may also imply the presence of two crystal populations in the oil medium. Microscopic examination indicated that this was the case in the presence of acetone. This is undesirable and should be avoided if a better crystallization technique is to be developed (5,6).

The melting behavior of canola sediment is also influenced by the concentration of sediment in the oil solution (1). Figure 3 shows a typical set of DSC thermal curves for melting of sediment in the mixture of hexane and canola oil. As the sediment content decreased, the melting endotherm shifted to lower temperatures, with a concomitant reduction in melting enthalpy. Figure 4 shows the corresponding DSC crystallization exotherms. These trends concur with our previous findings on phase transformation of canola sediment in oil and are consistent with classical solubility laws (15). Compared to the melting endotherms in Figure 3, the crystallization exotherms were usually characterized by a sharp peak and a shoulder or a sloping left side. With decreased sediment content, however, more symmetrical crystallization peaks were observed. Similar to the melting transitions, the crystallization peaks systematically shifted to lower temperatures with reduced enthalpies.

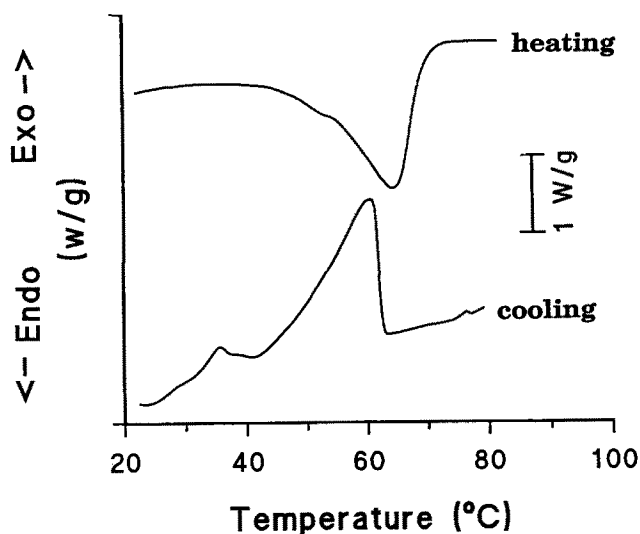


FIG. 2. Differential scanning calorimetry thermograms of sediment in canola oil and acetone mixture (1:1, w/w) at 20% (w/w) sediment content, obtained during a heating and cooling cycle, showing a melting endotherm and crystallization exotherms. Abbreviations as in Figure 1.

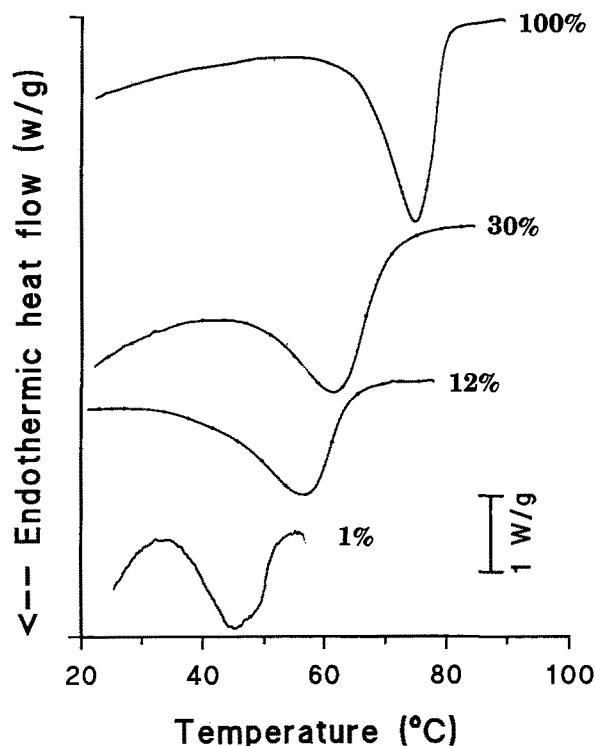


FIG. 3. Differential scanning calorimetry melting thermograms of sediment in canola oil and hexane mixture (1:1, w/w) at various sediment contents (% w/w).

Figure 5 compares the melting behavior of sediment in canola oil and solvents at 30% sediment content. The addition of a solvent decreased the melting temperature of sediment as evidenced by a shift in the melting peak. Hexane dramatically lowered the melting peak temperature by about 9°C compared to about 4°C with acetone. Figure 6 shows that the crystallization temperature of sediment in solvent/oil mixtures also decreased, as would be expected from the decreased melting temperature. In acetone/oil mixture, the decrease in crystallization temperature was much smaller (about 1°C) than in the hexane/oil system, which showed a temperature shift of the same magnitude to the change in melting transition (*ca.* 9°C, Fig. 5).

Figure 7 shows the DSC melting curves at 12% sediment content. The melting peaks all shifted to lower temperatures compared with the corresponding melting peaks of 30% sediment content (Fig. 5). The decrease in the melting peak temperature due to the inclusion of acetone was similar to that of 30% solids level, while the corresponding decrease in hexane was larger (10.5°C) than that of 30% sediment content. Figure 8 presents the corresponding crystallization curves at 12% sediment content. The crystallization temperature of sediment in hexane/oil was lower than in oil alone, consistent with the melting trend. However, the crystallization temperature of sediment in acetone/oil was higher than that in oil alone. This finding was somewhat unexpected considering the respective melting profiles where the peak melting temperature of sediment in acetone/oil did shift to a lower temperature than in

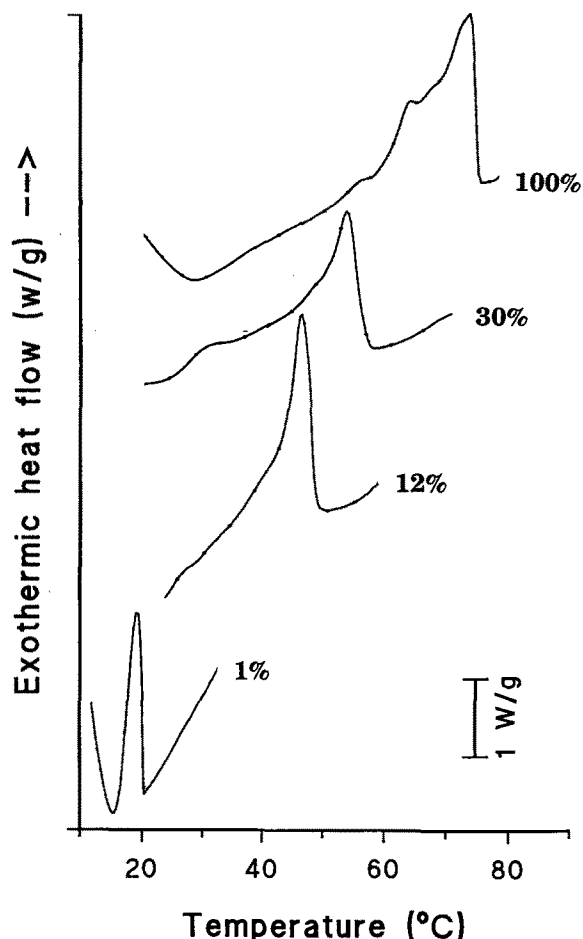


FIG. 4. Differential scanning calorimetry crystallization thermograms of sediment in canola oil and hexane mixture (1:1, w/w) at various sediment contents (% w/w).

oil (Fig. 7). The observation that the metastability of sediment in oil/solvent media, under dynamic conditions of cooling, is dependent on sediment content has prompted us to look at the phase transformation of this material over a wide range of sediment concentrations.

Figures 9 and 10 summarize the DSC melting and crystallization data, respectively. The peak melting temperature of sediment decreased as sediment content decreased (Fig. 9), with a more pronounced effect at low sediment contents (<10% w/w). Addition of acetone or hexane to canola oil substantially depressed the melting temperature of sediment, with a greater effect being observed with hexane. This means that, at a constant temperature, the amount of sediment dissolved in the liquid phase of oil/solvent mixtures is higher, i.e., a higher solubility of sediment occurs in such medium, and the sediment is less soluble in acetone than in hexane.

Figure 10 shows that the crystallization temperature of sediment in hexane/oil was always lower than in oil alone, consistent with the trend in the melting point depression. In acetone/oil, the crystallization temperature was slightly lower than in oil alone at high sediment contents. However, when the sediment content was low (<20% w/w), the crystalliza-

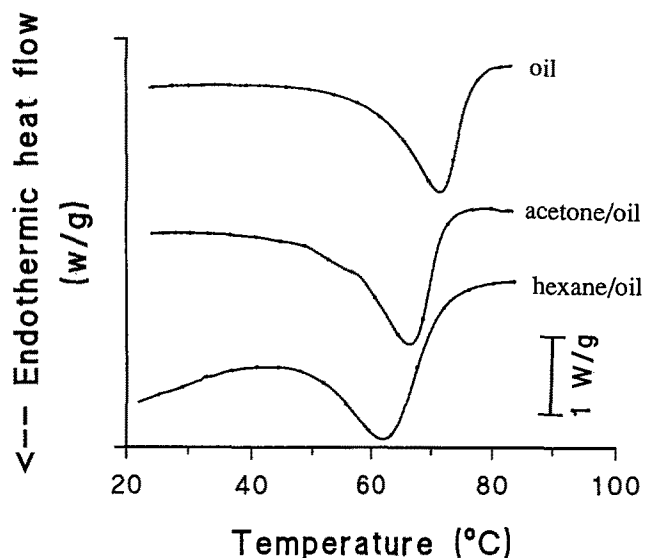


FIG. 5. Differential scanning calorimetry melting endotherms of sediment at 30% (w/w) sediment content in canola oil and oil/solvent mixtures (1:1, w/w).

tion peak occurred at higher temperatures than in oil (Fig. 10). Among other factors, crystallization is influenced by solubility and viscosity of the solution (14). When a solvent is mixed with oil, the solubility may increase, and this will reduce the crystallization temperature. Addition of a solvent also reduces the viscosity of the oil solution, thus facilitating the crystal-

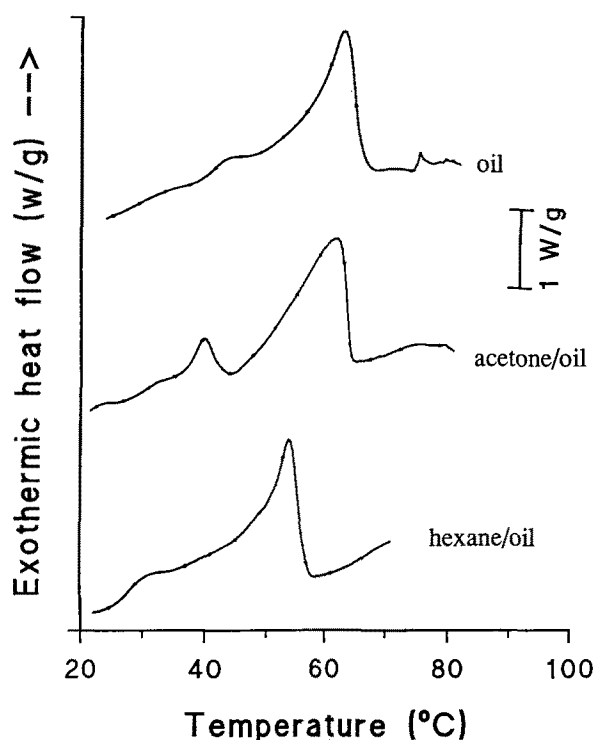


FIG. 6. Differential scanning calorimetry crystallization exotherms of sediment at 30% (w/w) sediment content in canola oil and oil/solvent mixtures (1:1, w/w).

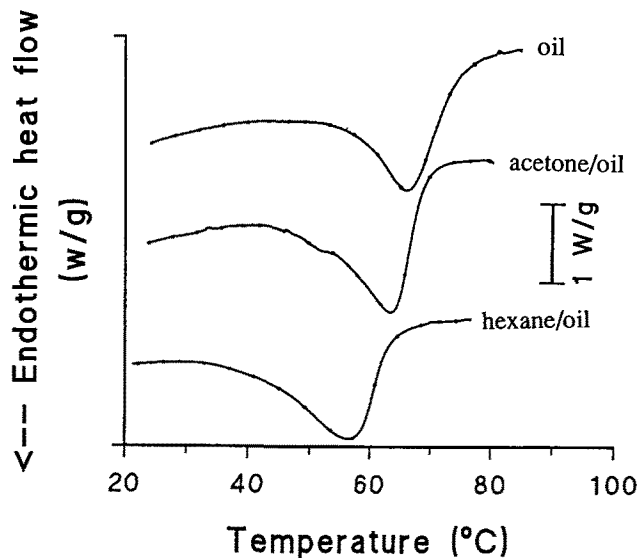


FIG. 7. Differential scanning calorimetry melting endotherms of sediment at 12% (w/w) sediment content in canola oil and oil/solvent mixtures (1:1, w/w).

lization process (8). Kapseu *et al.* (16) reported that the ratio of the viscosity of cottonseed oil and the pure solvents (acetone and hexane) is in the order of 300. Their results showed that the solvents added in the oil in 1:1 ratio (w/w) reduced the viscosity of the solutions by 60–70 times, with acetone reducing the viscosity more than hexane. The findings of Figure 10 suggested that the viscosity effect may be the domi-

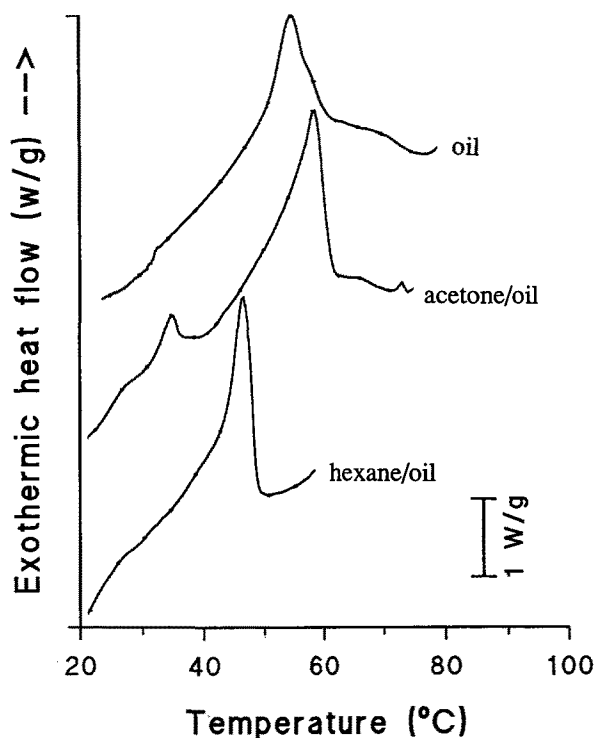


FIG. 8. Differential scanning calorimetry crystallization exotherms of sediment at 12% (w/w) sediment content in canola oil and oil/solvent mixtures (1:1, w/w).

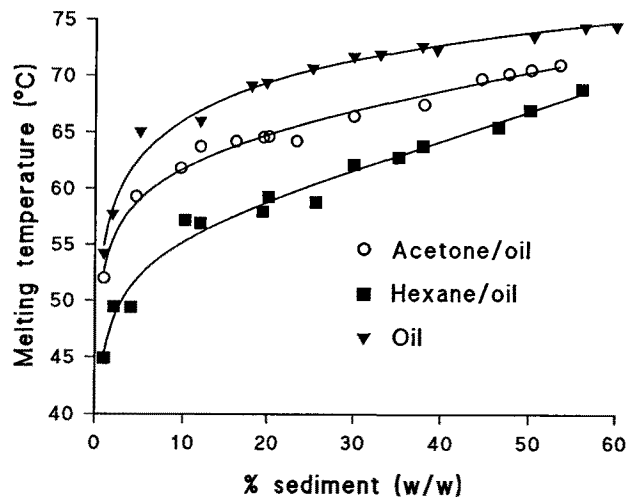


FIG. 9. Differential scanning calorimetry melting peak temperature of sediment in canola oil and oil/solvent mixtures (1:1, w/w), as a function of sediment content.

nating factor in acetone/oil solutions containing relatively low amounts of sediment.

Figure 11 summarizes the degree of supercooling in the three systems as a function of sediment content, where supercooling was estimated as the difference between melting and crystallization peak temperatures. In each case, supercooling increased with a decrease in sediment content. Supercooling was much smaller in the acetone/oil system than in the hexane/oil system and in oil alone. The supercooling in the mixture of hexane/oil was comparable with that in oil alone at high sediment contents and much higher in dilute solutions.

The enthalpy values, determined from the area under the DSC peak, decreased with decreased sediment content, particularly at low sediment concentrations (Fig. 12). Despite the differences in melting and crystallization behavior, however, the enthalpy for the phase transformation was virtually the

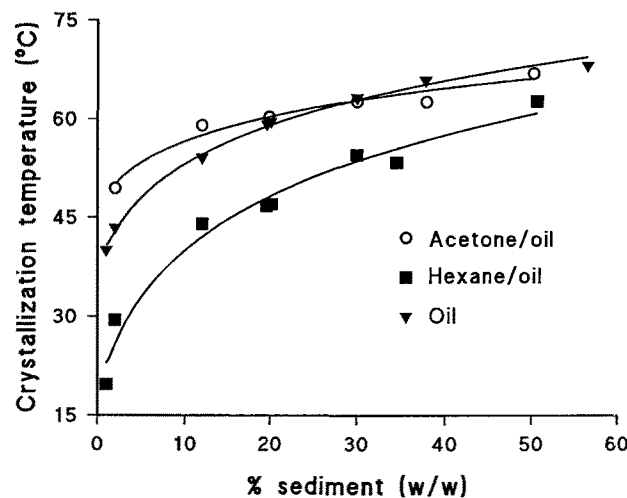


FIG. 10. Differential scanning calorimetry crystallization peak temperature of sediment in canola oil and oil/solvent mixtures (1:1, w/w), as a function of sediment content.

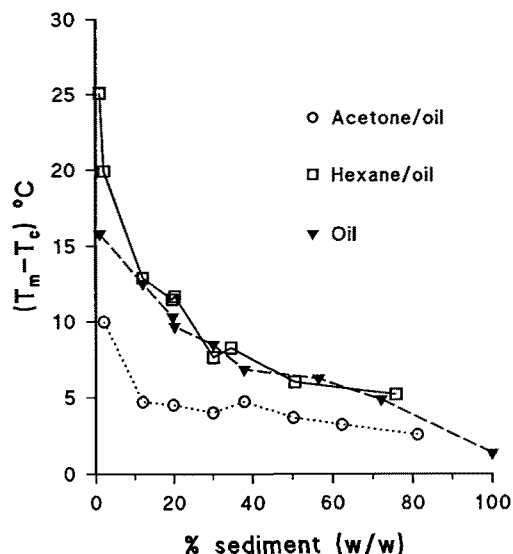


FIG. 11. Supercooling ($T_m - T_c$) in canola oil and oil/solvent mixtures (1:1, w/w) as a function of sediment content, where T_m is the melting peak temperature and T_c the crystallization peak temperature.

same for the three systems within experimental error. This would suggest that the solid phase formed in these systems was similar in supermolecular structure. The changes in the melting and crystallization characteristics are therefore merely a reflection of solubility and viscosity effects rather than polymorphism. This was also supported from X-ray diffraction data (1), which showed that, regardless of the crystallization conditions, canola sediment exists in a crystal structure, typical of waxes from different origins (1).

This study showed that solvents, such as acetone or hexane added to canola oil, increase the solubility of sediment in oil, with hexane exerting the greatest effect among the solvents tested. Because of increased solubility, the crystallization temperature in the mixture is generally lower. However, when acetone (not as good a solvent as hexane for sediment constituents) was used and at low sediment content, the crystallization temperature of sediment in the mixture was higher than that in oil alone, perhaps due to reduced viscosity of the solution. The results for acetone/oil mixtures indicated that an optimum ratio of solvent/oil must be used to facilitate the crystallization of high-melting waxy constituents of canola oil. Further studies on phase equilibria and phase transition behavior of sediment constituents in dilute oil/solvent mixtures are required to develop a suitable solvent system for turbidimetric assay of waxes in vegetable oils. The use of hot-stage microscopy, DSC, and X-ray diffraction, for probing crystal morphology and kinetics of phase transformations, would be essential in this respect.

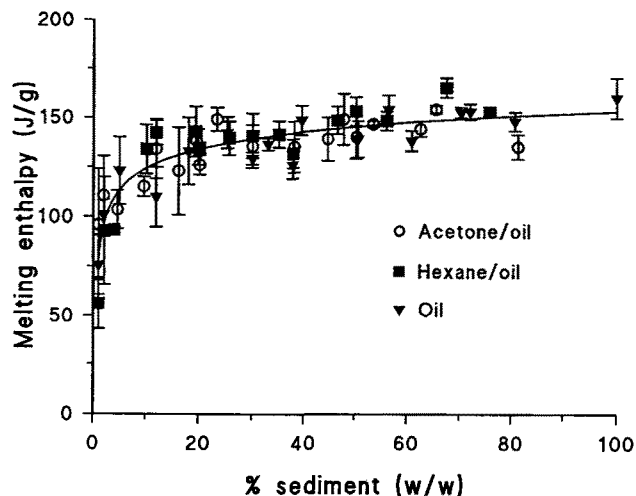


FIG. 12. Differential scanning calorimetry melting enthalpy of sediment in canola oil and oil/solvent mixtures (1:1, w/w) as a function of sediment content.

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