Rapid Determination of Wax in Sunflower Seed Oil

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ABSTRACT

A rapid turbidimetric method for determining wax content in sunflower seed oil is described. Oil is heated to 130 C, filtered, and after cooling, added to an equal volume of acetone. The mixture is then reheated under tap water to dissolve waxes which may have crystallized and is placed in an ice bath for 5 min. Turbidity is then measured and ppm wax is read from a previously prepared calibration curve. The amount of wax as determined by the turbidimetric method is in good agreement with the gas liquid chromatographic values.

INTRODUCTION

Waxes in sunflower seed oil are difficult to measure because of their low concentration (.02-.35%) in the oil (1). Several methods have been developed which give an accurate measurement of the wax content in sunflower oil (1-4). Each has its advantages and disadvantages. One gravimetric method which involves the use of an extraction procedure requires special equipment and long extraction times (1). Another method developed in this laboratory involves the gas liquid chromatographic (GLC) analysis of the alcohols hydrolyzed from the wax esters (2). Although it gives accurate results, it does not lend itself to rapid determination of wax content. A more rapid method developed by Brimberg and Wretensjo uses the turbidity of cold oil as a measure of wax content (3); however, it cannot be applied to crude oils. Caupeil (4) has developed a rather sophisticated method which uses a laser to detect microcrystalline formation in cooled oil. This, too, is a rapid method, but one which requires very specialized equipment. The method presented here is a modification of the Brimberg and Wretensjo method using some principles of solvent winterization to give a method that is rapid and applicable to crude and processed oils.

MATERIALS AND METHODS

A wax-free sunflower oil was prepared by dewaxing a refined (a process that also removes phospholipids) and bleached oil by cooling to 0 C for 24 hr and filtering under pressure in a previously cooled high-pressure filter containing a filter aid. This procedure was repeated 3 times. Pure wax was obtained by combining the above batches of filter aid and washing with cold hexane to remove residual oil followed by hot toluene to remove the waxes. After removal of toluene, pure wax was obtained by recrystallization of the residue from acetone. The wax-free oil and pure wax were used to prepare solutions of known wax content in oil.

Sunflower seed oil samples evaluated turbidimetrically ranged from crude to fully processed oils. Each oil sample was heated to 130 C to evaporate traces of water and was then filtered through Whatman # 4 filter paper to remove insoluble material. After cooling to room temperature, enough oil to fill to the mark was added to a 10-mL volumetric flask containing 5 mL acetone and the contents were thoroughly mixed. The oil/acetone mixture was transferred to a sample cell, stoppered, heated under hot tap water until clear, and placed in an ice bath for exactly 5 min. Turbidity was then measured in Nephelometric Turbidity Units (NTU) using a HACH Model 2100A turbidimeter which had been calibrated using formazin standards. Wax content was determined by referring to a previously prepared calibration curve. The calibration curve of ppm wax vs NTU was prepared from solutions of pure sunflower wax in dewaxed oil ranging from 44 to 2250 ppm. The samples used for the calibration curve were prepared as just described except that it was not necessary to heat the oil to 130 C prior to filtration.

To evaluate the length of time the oil would remain free from cloud formation upon refrigeration, 60-80 mL of the heated oil was placed in a clear glass bottle, sealed and refrigerated at 1 C. These samples were evaluated twice daily for clarity and for any sign of cloud formation.

A third sample of the heated oil was evaluated for wax content by GLC as previously described (2). All measurements were conducted in triplicate.

RESULTS AND DISCUSSION

In order to obtain an accurate measure of wax in sunflower oil by a turbidimetric procedure, complete or nearly complete crystallization of the wax is required. Winterization can be achieved more rapidly and completely if a mixture of acetone and hexane is added to the oil (2). Because waxes are relatively insoluble in acetone, the addition of acetone to oil was used to bring about nearly complete removal of waxes by crystallization. Brimberg and Wretensjo (2) suggested that turbidimetric measurements on crude oil would be difficult due to the presence of phospholipids which can retard crystallization. The use of acetone as described here made it possible to apply the turbidimetric method to crude sunflower oils. A 50:50 mixture of acetone and oil was placed in an ice bath and read after 5 min. Cloud formation was complete and uniform for all samples after 5 min; longer cooling times lead to erratic measurements due to settling and coagulation. With many samples, the mixture became cloudy as soon as oil was added to the acetone and if the sample were cooled in the ice bath and then read, poor results were obtained. It was discovered that if all samples were first heated under tap water until clear and then placed in the ice bath, readings were uniform and reproducible. As measurements were made quickly, crystal size was uniform and dispersed evenly throughout the solution, resulting in reproducible measurements.

Figure 1 shows that a plot of parts per million (ppm) added pure sunflower wax vs NTU deviates only slightly from linearity at high concentrations of wax. The quadratic equation $y = 1.68 + 0.56\,(x) + 1.3 \times 10^{-5} \times 2$ (y = ppm wax, x = turbidity in NTU) gives a correlation coefficient of .997, and is used to calculate wax content from turbidity measurements.

The results of turbidimetric and GLC measurements of waxes are compared and visual examination of the oils upon refrigeration are shown in Table I. In all samples, turbidimetric values for wax content are in good agreement with values determined by GLC. The average standard deviation for both GLC and turbidimetric values for wax on triplicate analyses was ± 10%.

Wax content measured by GLC for crude oils (samples 1-7) range from 345 to 2,020 ppm and are in fair agreement (± 10% deviation) with values of 415 and 1,795 ppm pre-

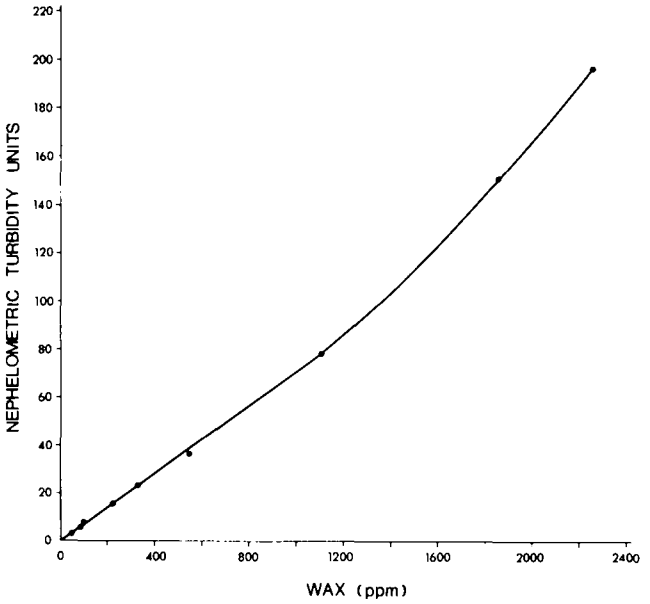

FIG. 1. Calibration curve. Nephelometric turbidity units (NTU) vs amount of added sunflower wax (0-2250 ppm).

TABLE I

Wax Content and Clouding Times for Sunflower Seed Oils

Sample	Treatment[b]	Wax (ppm)[a] GLC	Wax (ppm)[a] HACH	Clouding times (days)
1	C	345	415	<1
2	C	840	590	<1
3	C	1580	1575	<1
4	C	690	766	<1
5	C	1350	1482	<1
6	C	1325	1478	<1
7	C	2020	1795	<1
8	Sample 1, R	30	30	6
9	Sample 1, R, D	30	30	>7
10	Sample 2, R, B	480	414	<1
11	Sample 2, R, B, D	370	370	<1
12	R	0	26	4
13	Sample 12, D	0	24	4
14	R	533	677	<1
15	R	861	1047	<1
16	Commercial	80	26	>7
17	Commercial	75	66	>7
18	Commercial	41	30	>7
19	Commercial	55	52	3

[a]Wax ppm are ± 10% based on triplicate analyses.
[b]C, crude; R, refined; D, deodorized; B, bleached.

dicted by turbidimetric measurements.

Crude oils and oils at various stages of processing also show fair agreement between GLC and turbidimetric values. In nearly all cases, GLC values were slightly higher than turbidimetric values. This is probably due to incomplete crystallization of waxes.

Although it is not harmful to human health, waxes will form a cloudy precipitate upon refrigeration of the oil to give a product which is unacceptable to the American consumer. Seven days was arbitrarily picked as the number of days an oil of good quality would remain free of cloud formation during refrigeration at 1 C.

Although there appears to be good agreement between GLC and turbidimetric wax values, neither of these methods can be used to predict the wax concentration at which an oil sample will remain free of cloud formation. All crude oils become cloudy in less than 1 day and many do so as soon as they are cooled to room temperature. In this, as in other studies (2,3), as wax content dropped below 100 ppm, clouding times were greater, but no correlation could be obtained between wax content and clouding time, particularly at low wax content.

Samples 16-18 are commercial sunflower oil products which had undergone commercial dewaxing procedures and showed good clouding times. Note that samples 12, 13 and 19 have wax contents within the range of samples 16-18, but have clouding times of much less than 7 days. Apparently, there are changes brought about during processing that have a profound effect on cloud formation and these are under current investigation.

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