Difficulties in Physical Refining of Olive Oil, due to Presence of Triterpene 'Oleanolic Acid'

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ABSTRACT

Oleanolic acid was found to be responsible for the turbidity frequently observed in physically refined olive oil. General procedures for eliminating this problem and improving the final quality have been discussed. Special attention was given to optimizing the operating parameters of the deodorizer and keeping the rest of the physical refining process constant.

INTRODUCTION

The saponifiable fraction of olive oil contains traces of hydroxy-triterpenic acids. These are identified by thin layer chromatography (TLC) separation and gas chromatography/ mass spectroscopy (GC/MS) as maslinic acid (3β-2α-dihydroxy, 17-carboxy, Δ^{12}-oleanene), ursolic acid (3β-hydroxy, 17-carboxy Δ^{12}-ursene), betulinic acid (3β-hydroxy, 17-carboxy, Δ^{12}-lupene) and oleanolic acid (3β-hydroxy, 17-carboxy, Δ^{12}-oleanene (1). The chemical formulae are shown in Figure 1.

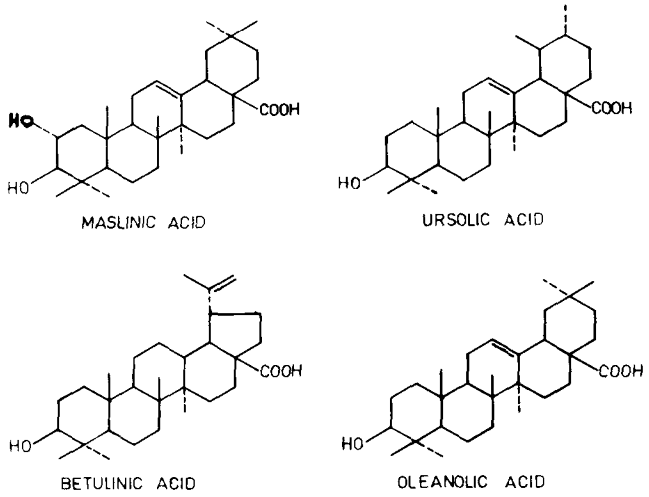

FIG. 1. Hydroxy-triterpenic acids in olive oil.

Attention should be concentrated on oleanolic acid among these terpenes for a better quality of refined oil. This triterpenic acid, which is present in higher ratios in olive husk oil than in pressed oil, has a good solubility in acidic medium. It frequently gives the refined oil a turbidity very similar to that of, e.g., inefficiently winterized sunflower seed oil. However, this turbidity is almost only seen in physically refined oils, obviously due to the omission of caustic soda usage in this process and elimination of the possibility of removing oleanolic acid as soap. The flow diagram of the physical refining process is shown in Figure 2.

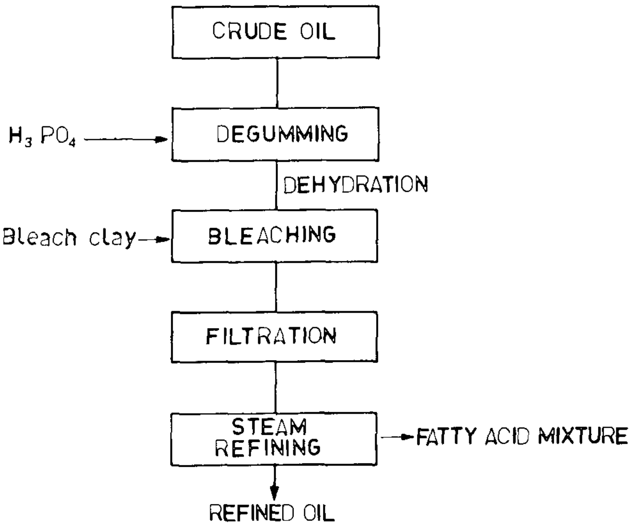

FIG. 2. Process flow of physical refining.

Oleanolic acid is present in olive leaves more than in the fruit itself and passes into the oil during pressing. Therefore, it is always critical to pick out the leaves and wash the fruit thoroughly before pressing.

Any of the following procedures can be applied in physical refining for overcoming the turbidity seen in refined oils containing considerable amounts of oleanolic acid. (a) The slightly heated crude oil is kept for a long period in storage tanks. This way, it is expected that oleanolic acid separates and settles down as "foots". (b) The degummed and bleached oil is first passed through the deodorizer to reduce the free fatty acids (FFA) down to ca. 1%. This is mixed with 10% crude oil in order to increase the solubility of oleanolic acid. Then the rest of the FFA are neutralized with caustic soda and the oil is passed through the deodorizer once more. (c) The degummed and bleached oil is submitted to winterization and subsequently filtered.

(b) and (c) of the above procedures lower the capacity and cause an additional loss of neutral oil. The disadvantage of (a) is loss of time due to foots settling down in the storage tank.

In our case, however, the turbidity was observed only occasionally and with crude oil coming to the plant there was an obligation to work continuously. These two reasons prompted us to seek a more practical solution for elimination of this undesirable effect.

EXPERIMENTAL

The first time we encountered turbidity was during the crop of 1978/1979. The results of simple laboratory tests with both crude and refined oil, which directed our investigations toward oleanolic acid, can be summarized as follows. (a) The turbidity disappeared at 40-45 C and reformed when cooled to 20-22 C. (b) Analysis by GC gave the usual saturated fatty acid content. (c) The turbidity could not be filtered using an ordinary laboratory filter paper. (d) After a certain period of time, a flaky precipitate formed from the turbidity, settling very slowly. (e) When 5% of a pure fatty acid mixture of olive oil was added to the refined oil at 45 C and cooled down, a slight turbidity could be observed only after 2-3 days. (f) However, the turbidity was completely eliminated upon alkali treatment.

Finally, we centrifuged the turbid oil, washed the precipitate several times with hexane for quantitative removal of traces of oil and recrystallized from methanol/hexane in which it was rather soluble. Comparison of the infrared (IR) spectrum taken on this product with that from literature (2) confirmed our suspicion and showed the compound to be oleanolic acid (Fig. 3).

Once the problem-causing compound was identified, the next step was the development of a method enabling its detection in the crude oil before it is sent to the refinery. This way, the batches with a high oleanolic acid content can be stored in separate tanks to be refined separately afterwards.

First we tried to determine normal oleanolic acid content in oil. Crude oil was kept at 0 C for 24 hr in hexane 5 times its weight and the concentration was determined from the portion insoluble in the solvent (2). This, however, did not give reproducible results. Therefore, instead, we determined the triterpenic alcohol content of the crude oil. The concentration of triterpenic alcohols, which make up a high percentage of the total unsaponifiable matter and have the same basic chemical structure as the acid (Fig. 4), was expected to increase as the oleanolic acid concentration increased. This fact is illustrated by the data in Table I, derived from only 10 of our determinations.

Determination of Triterpenic Alcohol Content

The unsaponifiable fraction was prepared according to AOCS Method Ca 6a-40, using a 10 g sample, and was dissolved in 2 mL of chloroform. Using this solution, 15-20 mg of the unsaponifiable matter was applied to preparative-TLC plates (Merck, Silica Gel 60, 20 X 20 cm, layer thickness 2 mm) in a continuous line of droplets with the help of a microsyringe. The plates were developed in a mixture of hexane/diethyl ether (80:20). The resolved bands were made visible by spraying the plates with a very dilute solution (ca. 0.1%) of 2,7-dichlorofluorescein in ethanol. The triterpenic alcohol band was carefully scraped off the plate into a conical flask and was boiled under reflux with 10 mL of chloroform. Silica gel was filtered off, the solvent evaporated and the weight of the alcohol mixture was accurately determined.

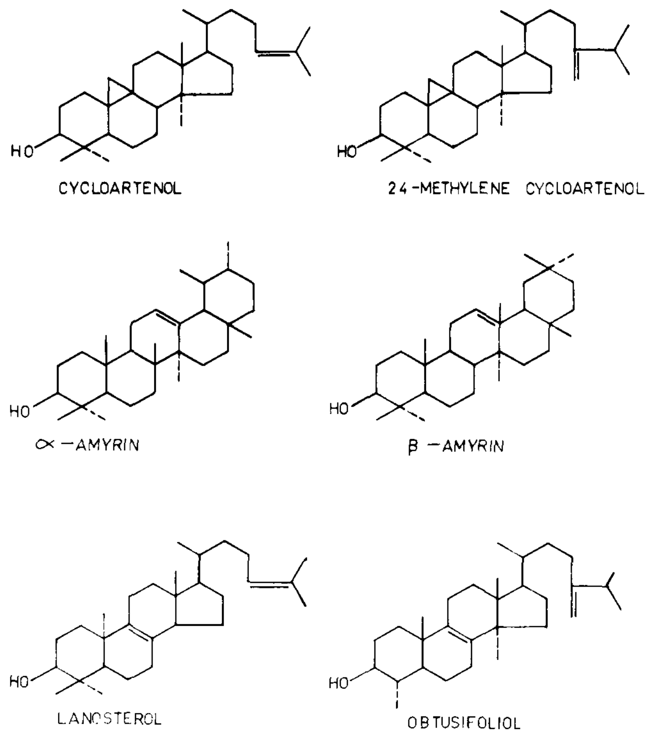

FIG. 4. Triterpenic alcohols in olive oil.

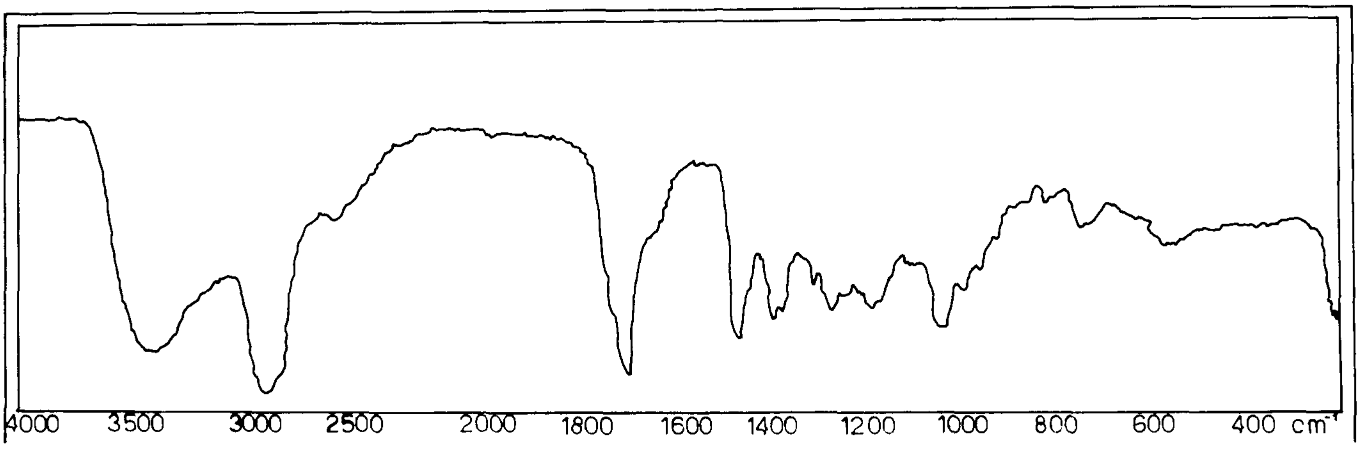

FIG. 3. IR spectrum of oleanolic acid.

Unfortunately, the method was quite time-consuming, preventing its routine application to every crude oil delivery. However, during our examination of the crude oils from Karpuzlu and other Çine areas, we have noticed that the turbidity was visible immediately after the oil had been bleached. We still do not have a reasonable explanation for this fact, but we have decided to utilize this and adopt it as a simple test. We believe that crude oils with a high oleanolic acid content can be detected with ca. 90% reliability using this test.

Following the detection of batches with a high oleanolic acid content, a practical and a reliable procedure had to be developed for their deodorization and deacidification steps in order to eliminate turbidity in the refined oil. Our studies in a laboratory pilot plant (Fig. 5), where oil samples with different contents of oleanolic acid were processed under typical conditions of commercial operation, have helped us considerably in finding a satisfactory solution. We realized that by altering and optimizing the deodorization parameters such as vacuum, temperature, amount of stripping steam and oil feeding rate, it was possible to remove a high portion of oleanolic acid together with the FFA. To illustrate this, we determined the triterpenic alcohol content of the FFA of refined oils having no turbidity, i.e., originating from crude oils which remain very clear upon bleaching and of those showing no turbidity only when the operation parameters were under full control. The percentages of triterpenic alcohol in the unsaponifiable portion of the FFA (indirectly representing the oleanolic acid content) are shown in Table II.

TABLE I

Triterpenic Alcohol Content of Crude and Refined Oils

Oil	Unsaponifiable (%)	Triterpenic alcohols (% of unsaponifiable)	Triterpenic alcohols (% of oil)
Crude (Karpuzlu)	0.80	60.10	0.48
Crude (Karpuzlu)	0.58	59.20	0.35
Crude (Çine)	0.69	77.61	0.96
Crude (Çine)	0.74	58.40	0.54
Crude (Milas)	1.20	18.60	0.22
Crude (Milas)	0.79	16.18	0.13
Crude (Milas)	1.10	21.97	0.24
Crude (Aydin)	0.92	19.42	0.18
Physically refined (turbid)	0.80	44.00	0.31
Physically refined (turbid)	0.72	39.30	0.28

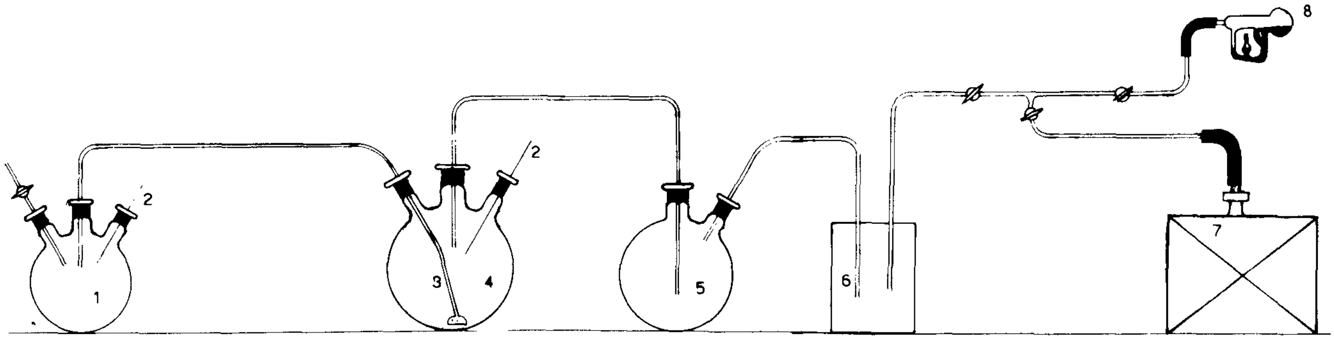

FIG. 5. Laboratory pilot plant. (1) Steam generator; (2) thermometer; (3) steam distributor; (4) deodorizer; (5) fatty acid condenser; (6) freezing trap; (7) vacuum pump; (8) McLeod manometer.

TABLE II

Triterpenic Alcohol Content of the Distilled Fatty Acids

	Unsaponifiable (%)	Triterpenic alcohols (% of unsaponifiable)	Triterpenic alcohols (% of distilled fatty acid)
Distilled fatty acids[a]	5.45	1.80	0.098
Distilled fatty acids[b]	6.30	2.98	0.189

[a] Obtained while refining crude oil blend from the Milas and Aydin areas only.

[b] Obtained while refining crude oil blend from the Karpuzlu, Çine, Milas and Aydin areas. Deodorizing parameters optimized.

RESULTS AND DISCUSSION

It seems that determining the triterpenic alcohol content of the unsaponifiable matter gives reliable results regarding oleanolic acid content. Table I compares the triterpenic alcohol concentration of crude oil blends from the Karpuzlu, Çine, Milas and Aydin areas, determined as a percentage of the unsaponifiable matter. It is seen that crude oil from Karpuzlu and Çine contains the highest concentrations (58.4% minimum and 77.61% maximum), while those from Milas and Aydin contained considerably lower amounts (21.97% maximum). This was somewhat expected, since we first observed turbidity when our crude oil blend contained a high percentage of oil from Karpuzlu/Çine for the first time. On the other hand, taking into consideration that our main blend consisted of oil from these 4 areas alone, it is quite obvious that the triterpenic alcohol contents (44 and 39.3%) of the 2 refined oils, which both showed turbidity, lie somewhere between those from Çine/Karpuzlu and Milas/Aydin.

Oleanolic acid contents of the olive leaves and of the fruit itself vary from crop to crop. For example, Karpuzlu and Çine areas delivered the most problematic oils from the crop of 1978/1979, whereas during the next crop, crude oils from none of the regions around Mugla caused any trouble. In January 1982, however, oils from the Aydin area have also been found to contain a considerable amount of oleanolic acid.

The appearance of turbidity in the crude oil upon laboratory scale bleaching is considered to be of particular value in distinguishing the batches that need to be deodorized separately.

The turbidity in the deodorized olive oil was eliminated by controlling certain parameters in the deodorizing unit. It was found that reducing the free acidity down to a certain limit was always necessary to remove the turbidity in the final oil. In this way, the distilled fatty acids were enriched in oleanolic acid. This is illustrated in Table II, where the triterpenic alcohol contents of the unsaponifiable fractions of the fatty acids are given. Fatty acids obtained during refining of a crude oil blend only from the Milas and Aydin areas showed a value of 1.80%, whereas fatty acids of the blend containing oil from the Karpuzlu and Çine areas as well showed a value of 2.98%. Experiments on the laboratory pilot plant have indicated that this value would lie in the range of 2-2.5% if the free acidity was not brought down to a certain limit, in which case the refined oil would show turbidity.

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