# Letters to the Editor

## Detection of B-Residue Oil in Commercial Olive Oils

*Sir:*

A recurrent question in the analysis of fats and oils concerns the detection and identification of B-residue or pomace oil in commercial olive oils. B-residue is that oil which is solvent-extracted after the olives have been pressed to obtain "virgin" oil. The method suggested by Eisner et al. (1) was investigated and found to be a reliable indicator of B-residue oil. Results of this investigation plus a few modifications and insights into the procedure are given below.

An examination of the literature quickly reveals that fatty acid compositions are not reliable indicators of adulteration of olive oils. Therefore, components in the unsaponifiable fraction were chosen for differentiation (1). Unsaponifiable matter was obtained from olive oils by the conventional technique of liquid-liquid extraction of saponified samples as described in the Official and Tentative methods of the American Oil Chemists' Society (AOCS), 3rd Edition, Method 6a-40. A second, more facile procedure was developed whereby the unsaponifiables were separated from the soaps by filtration.

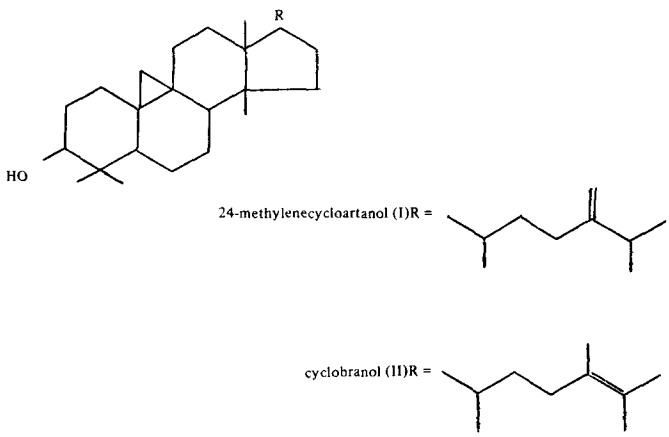
For this procedure, 2 g of oil was refluxed in 3 ml of 50% KOH and 30 ml of ethanol in a 100 ml round-bottomed flask for 2 hr. The solution, after cooling, was concentrated on a rotary evaporator until clumps of soap formed and no more distillate was forthcoming. A few ml of acetone was added, the clumps were broken up with a stirring rod, and it was again evaporated to dryness. (The addition of acetone and evaporation could be repeated as deemed necessary to remove water from the system.) After the final evaporation, 50 ml of ethyl ether was added, and the clumps were again broken up. This slurry was swirled for a few minutes and filtered through Whatman No. 5 filter paper. More ether was added, swirled, and filtered until about 75 ml of filtrate was obtained. The solvent was removed under $N_2$ on a steam bath. The residue was taken up in about 200 µl of $CHCl_3$ and analyzed directly by gas chromatography (GC) on a 6 ft x 2 mm ID glass column packed with 3% OV-1 on 100/120 mesh Gas Chrom Q in a Packard 7401 chromatograph equipped with a flame ionization detector. The injection port was 240 C, the detector oven 280 C, and the column oven was temperature programmed from 210 C to 270 C at 2 C/min. Typically 2-3 µl was injected. Helium was the carrier gas at 40 psig (30 ml/min).

Three authentic sets of samples, including one each of virgin (pressed oil), refined virgin, and B-residue oils, were analyzed along with six samples (A-F) of unknown origin.

Persistent emulsions are easily formed and can cause problems during the isolation of unsaponifiable matter by the conventional AOCS method. The filtration technique appeared to be an appropriate alternative because it was fast, easy, and gave GC curves nearly identical to those of the AOCS-derived unsaponifiables.

The procedure developed by Eisner and coworkers (1) involves finding the ratio of the peak areas of two (or more) triterpene alcohols. In this way, detector responses are more or less empirically derived, and the components are not determined absolutely. Determination of the individual responses of the alcohols would probably be the method of choice, but they are not readily available in high purity.

A key factor in recognizing B-residue oil involves a hypothesis as to why its triterpene alcohols appear different from those of virgin or refined oils. A significant component of olive oils is 24-methylenecycloartanol (I), which has been shown to rearrange to cyclobranol (II) under acidic conditions (2). Other minor byproducts are also formed.




Perhaps enough energy (heat) is supplied or other important conditions are met during the pressing and/or extraction of olives to cause this isomerization to proceed. In any event, cyclobranol has been found in certain olive oils (3).

(I) was arbitrarily assigned a RRT (relative retention time) of 1.00; (II) then gave an RRT of 1.2. A third unknown component (III, RRT = 0.4) also occurred in B-residue oils at much higher levels than in virgin or refined oils.

The data in Table I show that area ratios for I/II are above 100 for virgin oils, range from 25 to 35 for refined oils, and are below 6 for B-residue oils. Ratios for I/III are not so uniform but can be approximated as >15 for virgin oils, 5-15 for refined oils, and <2 for B-residue oils. No attempt was made to measure the statistical reliability of this method, and some differences in the data are evident. However, the ranges for the three oil types are consistent and can be used to infer the sources of the unknown samples. Samples B through F are probably refined oils or are mixtures of refined and virgin oils. Unknown A would appear to be a mixture of refined and B-residue oils, approximately 50:50. This conclusion was arrived at by applying the lowest value for refined oil (25) and the highest for B-residue (6). The calculation is 25 x 0.50 + 6 x 0.50 = 15.5. Although a broad spectrum of standard mixtures was not made and analyzed, adulteration at the 10% level should be detectable (25 x 0.90 + 6 x 0.10 = 23.1). Olive oils with ratios (I/II) of less than 25 should be suspect.

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TABLE I

Peak Area Ratios for Standard and Unknown Olive Oils

| Sample | Unsap method[a] | Peak area ratio I/II | I/III |
|---|---|---|---|
| Authentic Virgin 1 | Conventional | ∞ | 16 |
| Authentic Virgin 1 | Filtration | ∞ | 82 |
| Authentic Refined 1 | Conventional | 36 | 6.3 |
| Authentic Refined 1 | Filtration | 27 | 7.6 |
| Authentic B-residue 1 | Conventional | 6 | 1 |
| Authentic B-residue 1 | Filtration | 2.6 | 0.8 |
| Authentic Virgin 2 | Filtration | 139 | 48 |
| Authentic Refined 2 | Filtration | 27 | 13 |
| Authentic B-residue 2 | Filtration | 5 | 1.5 |
| Authentic Virgin 3 | Filtration | 124 | 20 |
| Authentic Refined 3 | Filtration | 25 | 8.3 |
| Authentic B-residue 3 | Filtration | 5.2 | 2 |
| Unknown A | Filtration | 14 | 2.5 |
| Unknown B | Filgration | 32 | 10 |
| Unknown C | Filgration | 30 | 9 |
| Unknown D | Filtration | 66 | 6 |
| Unknown E | Filtration | 32 | 7 |
| Unknown F | Filgration | 43 | 14 |

[a]Conventional = AOCS Method; filtration = NRRC-designed method.

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# Stabilization of Rice Bran with Sodium Metabisulfite

*Sir:*

The rapid rise of free fatty acids in fresh rice bran is well known. There are methods to control this activity, which has been attributed to the presence of lipase enzyme in rice bran. Methods that are used are cold storage and heat stabilization, by which the lipolytic activity in bran is either immobilized or destroyed. Both methods have their merits and limitations. A third alternative, namely, chemical stabilization of rice bran, has been tried. We selected sodium metabisulfite which is known to be a good preservative used in food industries. It is also used as a disinfectant and an antiseptic. Thorough mixing of rice bran and chemical is essential, and for this purpose a laboratory and a pilot plant method were devised. In the laboratory, trials were carried out by mixing fresh rice bran and sodium metabisulfite with a mortar and pestle. The optimum level of the chemical that is effective in stabilizing the bran was found to be 2% the weight of bran. The additive-treated sample and the original untreated rice bran (control) were stored in stoppered glass bottles simultaneously. Samples were drawn from both bottles periodically; oils were extracted and free fatty acids of the oils were determined by official AOCS Methods. After observing the effectiveness of the chemical, a more vigorous and continuous mixing device was tried in scaled up studies. Here an expeller was selected for effecting a thorough and intimate mixing of bran with the chemical. The feed was well mixed while being propelled forward under pressure through worm helicals. Twenty-five kg of fresh rice bran admixed with 500 g of sodium metabisulfite was run cold through the screwpress. Similar quantity of fresh rice bran without additive was treated the same way. The two treated samples were stored simultaneously in jute bags. Samples were drawn periodically, oils were extracted, and free fatty acids of oils determined.

It was found that the free fatty acid in oils from untreated rice bran stored for 0, 10, 20 and 30 days were 2.2, 12, 20 and 24, and the corresponding figures for chemically treated rice bran were 2.2, 2.5, 2.5 and 3.5, respectively. It would appear that chemical treatment had immobilized lipase activity in the bran and stabilized it for safe storage over a period of one month. It was also observed that the oil yield from bran was not reduced by treatment with the chemical. The low ffa oil obtained from the additive-treated rice bran could be subjected to the usual refining processes and the refined oil thus obtained would be suitable for edible purposes. Work needs be done on the utility for extracted meal obtained from chemically treated bran.

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