DETECTION OF TRIETHYL CITRATE AND TRIACETIN IN LIQUID AND DRIED EGG WHITES[1]

L. KOGAN AND S. STREZLECK

ABSTRACT

A method is described for detection and identification of the whipping aids triethyl citrate and triacetin in egg whites. The sample, either liquid whites or dried whites that have been reconstituted, is extracted with diethyl ether. The presence of the whipping aid in the residue of the ether extract is established by gas-liquid chromatography. Unequivocal identification is made by infrared spectroscopy, using the gas-chromatographically purified material to obtain the infrared spectrum.

Triethyl citrate (1) and triacetin (2) are additives used in egg whites to improve the whipping properties. When added to liquid or dried egg whites, these compounds markedly decrease the time required for whipping the egg whites to a uniform, stable foam. Normal use levels for these additives are quite low (about 0.025% for triethyl citrate and about 0.15% for triacetin, both on a liquid egg white basis). The procedure described herein was developed to fill a need for a more reliable means of detecting and identifying these egg white additives. Prior to the development of this procedure, these additives were identified after extraction from the egg white with diethyl ether by simple qualitative tests. Triethyl citrate was characterized as an ester by the hydroxamic acid test. In this test, the ester is converted into the sodium salt of the corresponding hydroxamic acid by reaction with hydroxylamine hydrochloride in the presence of sodium hydroxide. This in turn reacts with ferric chloride in the presence of acid to produce a red or violet-colored ferric salt. The ester is further identified as being derived from citric acid by the citrizinic acid test (the formation of a fluorescent blue ammonium salt of citrizinic acid). The correct refractive index and exhibition of the characteristic bitter taste completed the identification. Similarly, triacetin was identified by the ester test, a qualitative test for glycerol (acrolein test), by refractive index, and by checking for the acetic acid moiety after hydrolysis.

Materials and Methods

Gas-Liquid Chromatography. The instrument used was the Chromacon, model 9475-35 (made by Podbielniak, Inc.) with thermal con-

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ductivity detector. The instrument was modified so that the detector could be operated with a bridge current of 200–300 milliamp., thus greatly increasing the sensitivity. (The instrument was designed to operate with a bridge current of 50 milliamp.) A heater was installed around the column outlet to prevent excessive premature condensation of the vapors eluted from the column in this portion of the apparatus. This facilitates collection of the purified condensed triethyl citrate and triacetin vapors in the collection bottle. Condensation of the eluted vapors in the column outlet prior to collection of the purified sample is undesirable, since it would permit contamination of the sample and defeat the purpose of the gas-chromatographic purification.

The column used was ¼-in., 6-ft. copper tubing packed with Chromosorb-W (30/60-mesh) coated with silicone gum rubber (SE-30). To prepare the column packing, 20 g. of the silicone gum rubber was dissolved in sufficient chloroform to cover 100 g. of the Chromosorb. After thorough mixing, the chloroform was evaporated off on a steam bath before the column was packed.

Operating temperatures were (°C.): sample injection block, 250; column, 200; column outlet heater, 165.

The carrier gas was helium at a pressure of 10 p.s.i.

Spectroscopic Measurements. Infrared spectral identifications were obtained on thin films (on salt plates) of the purified material collected at the column outlet of the gas chromatograph. The instrument used was the Perkin-Elmer, model 137.

Detection of Triethyl Citrate and Triacetin. Extraction. The liquid egg whites were extracted with diethyl ether (dried egg whites were reconstituted at a ratio of 7.15 g. to 50 ml. of water prior to the ether extraction). Triethyl citrate is more readily extracted if the pH of the liquid whites is adjusted to 3.0 with concentrated H_2SO_4 prior to extraction. Because triacetin hydrolyzes readily at pH 3 to yield glycerol and acetic acid, ether extraction of this additive must be made on unacidified egg white. This means that two extractions must be carried out on unknown egg white samples: 1) on the acidified sample to determine triethyl citrate, and 2) on the unacidified sample to determine triacetin.

It is usually necessary to extract 500 ml. of liquid egg white to obtain sufficient material to complete the identification. About 1 liter of diethyl ether was required for the extraction, which was carried out in a large separatory funnel. Successive 100-ml. portions of the liquid egg white were shaken with the same 1-liter portion of ether. This was accomplished by drawing off most of the first 100 ml. of sample after it was shaken with the ether. The second 100 ml. was then

introduced into the separatory funnel, shaken, and allowed to separate; the egg white layer was then drawn off and discarded. This process was repeated until the entire 500 ml. of sample had been extracted. The ether layer was filtered through glass wool to separate any remaining albumen. The glass wool was washed with additional diethyl ether. The ether used to wash the glass wool was combined with the ether extract of the sample and concentrated by evaporation to about 1 ml. under a stream of nitrogen. Presumptive evidence of the presence of triethyl citrate or triacetin was obtained by gas chromatography of 5 μl. of this concentrated extract. Under the above operating conditions, the peak for triacetin appeared after about 8 min. and that for triethyl citrate after 23 min.

Purification of Extract and IR Identification. If a positive presumptive test was obtained, the remainder of the ether extract was evaporated under a stream of nitrogen until all of the ether was removed. Twenty-five microliters of the oily residue was placed on the column. When the recorder began to trace the peak associated with the additive in question, a small vial was placed over the outlet of the column and the condensing vapors were collected. To cool the vial slightly, it was wrapped with a wet cloth. The condensate was collected as long as the recorder continued to trace the peak. Infrared spectra were obtained by dissolving the condensate in a small volume of anhydrous diethyl ether and transferring a thin film to the salt crystal by evaporating a drop of the solution on the crystal.

Results and Discussion

The procedure was tested by adding triethyl citrate and triacetin at use levels to separate samples of liquid egg white. After extraction and gas-chromatographic purification, characteristic IR spectra were obtained for triethyl citrate and triacetin. Figure 1 shows the IR spectrum of triethyl citrate and of the triethyl citrate isolated from the egg white. The IR spectra for triacetin and for the triacetin extracted from the egg white sample are shown in Fig. 2.

A commercial sample of dried egg white known to contain triacetin was examined by the procedure described. The preliminary gas chromatography indicated that the sample contained triacetin plus an impurity. The IR spectrum for the purified extract was similar to that for triacetin but slightly different. When the column temperature was reduced to 150°C., the triacetin separated sufficiently from the impurity to be collected. Infrared spectroscopy of this purified material gave a characteristic spectrum for triacetin. The retention time for the impurity indicated that it was probably diacetin. Since the peak for

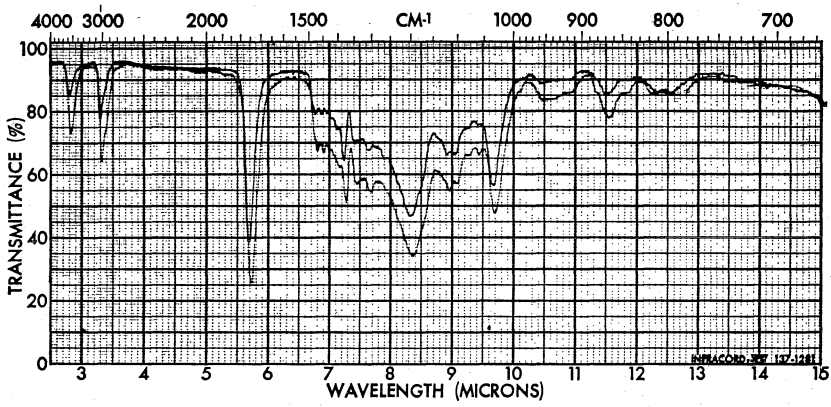

Fig. 1. Upper curve, infrared spectrum of triethyl citrate extracted from egg white; lower curve, infrared spectrum of triethyl citrate.

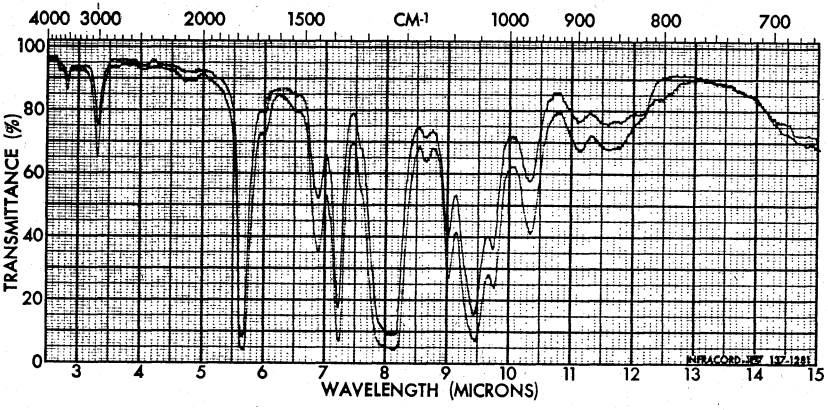

Fig. 2. Upper curve, infrared spectrum of triacetin extracted from egg white; lower curve, infrared spectrum of tracetin.

this impurity appeared before the triacetin peak and since it was present at a very low level relative to the triacetin, no further effort was made to confirm the identity of the impurity.

The procedure is recommended only for its qualitative applications. No recovery experiments were carried out during the development of this procedure, because our experience with the extraction of liquid and reconstituted dried egg whites for colorimetric assays by the hydroxamic acid test has shown that only about 50 to 85% recoveries of triacetin and triethyl citrate may be expected when quantities as large as those required for this test are extracted. We have, however, experienced no difficulty in detecting these additives by this

procedure when present at effective use levels. In our experience as little as 0.2 to 0.3 mg. of triethyl citrate or triacetin is ample to obtain infrared spectra similar to those shown in Figs. 1 and 2. The recoverable amounts of triacetin and/or triethyl citrate extracted from the egg whites containing use levels of the respective additives are greatly in excess of the IR requirements.

Acknowledgment

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