

A New Quantitative Procedure for Determination of Sinapine

A new colorimetric method is described for determining sinapine based on the colored complex formed with titanium tetrachloride. Determination of the sinapine content of rapeseed flour and protein concentrates demonstrated the reliability of this procedure over the Reinecke salt method due to the stability of the titanium-sinapine complex.

Sinapine, a choline ester of sinapic acid, has been reported in seeds of *Brassica napus* and *B. campestris* (Clandinin, 1961; Austin and Wolff, 1968) and *Crambe abyssinica* (Austin and Wolff, 1968). Recently, Hobson-Frohock et al. (1977) implicated sinapine in rapeseed meal with the production of a "fishy" taint in brown-shelled eggs. A standard method for estimating sinapine is based on complex formation with Reinecke salt (Austin and Wolff, 1968; Tzagoloff, 1963); however, this paper reports a new quantitative procedure based on the formation of a colored complex between sinapine and titanium tetrachloride. This reagent has been shown earlier in our laboratory to complex with phenolic compounds (Eskin et al., 1978).

EXPERIMENTAL SECTION

Reagents. Sinapine bisulfate (mp 187–190 °C), isolated and purified from rapeseed, was provided by Dr. D. R. Clandinin, University of Alberta, Canada. Titanium tetrachloride and Reinecke salt (ammonium tetrathiocyanodiammonochromate) were purchased from British Drug Houses (Toronto Canada) and Fisher Scientific Co., NJ, respectively. Defatted rapeseed flour and rapeseed protein concentrates were provided by Dr. John Jones, Food Research Institute, Ottawa, Canada.

Procedure. *Preparation of Sinapine Standard.* A series of standard solutions was prepared for sinapine (0–500 µg/mL) in 10 mL of concentrated HCl in 15-mL screw-top glass tubes. To each tube was added 0.25 mL of titanium reagent (20% TiCl₄ in concentrated HCl), and the solutions were thoroughly mixed in a vortex for 15 s. A colored complex formed immediately, and the absorbance spectra examined using an SP 800 Unicam spectrophotometer over 400–550 nm against an equivalent blank.

Extraction of Sinapine from Defatted Rapeseed Flour and Concentrates. The sinapine content of defatted rapeseed flour and protein concentrates was examined using the titanium reagent. Sinapine was extracted from rapeseed flour and protein concentrates as outlined in Scheme I. It was necessary to extract with acetone-water as this proved to be more efficient than extraction with either ethanol or methanol. In order to ensure no losses occurred during the extraction procedure, a known sample of sinapine was added to samples of rapeseed flour and protein concentrates and extracted under identical conditions. For comparison sinapine content was also determined using the Reinecke salt method (Austin and Wolff, 1968; Tzagoloff, 1963).

RESULTS AND DISCUSSION

The spectrophotometric data for sinapine-titanium tetrachloride complex is shown in Table I. Molar absorptivity, however, is only approximate. A linear Beer's law plot was evident by the coefficient of determination (r^2) value of 1.00. The stability of the colored complex was linear over 24 h which is a distinct advantage over the Reinecke salt method in which the color is very unstable.

The sinapine content of defatted rapeseed flour and concentrates using the titanium and Reinecke salt methods

Scheme I. Extraction of Sinapine from Defatted Rapeseed Meal and Its Determination by Titanium and Reinecke Salt Methods

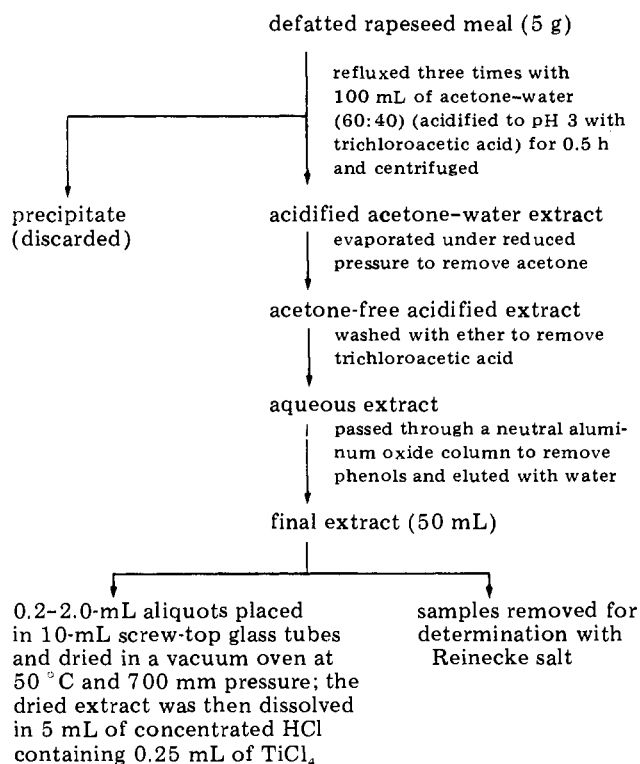


Table I. Spectrophotometric Data for Sinapine-Titanium Tetrachloride Complex

compd	max, nm	molar absorp.	measured at nm	Beer's law plot (r^2)
sinapine	485	5.67×10^2	485	1.00

Table II. Sinapine Content of Rapeseed Flour and Concentrates by Titanium and Reinecke Salt Methods^a

sample	sinapine, %	
	titanium method	Reinecke salt method
rapeseed flour	1.04 ± 0.03	1.04 ± 0.14
rapeseed protein, concentrate I	0.18 ± 0.01	0.20 ± 0.03
rapeseed protein, concentrate II	0.11 ± 0.01	0.12 ± 0.04

^a Determination carried out in triplicate.

is tabulated in Table II. Similar results were obtained using both procedures; however, the larger standard deviations observed using the Reinecke salt reflected the instability of the colored complex formed. The absorbance spectrum of the extracted sample with titanium tetrachloride was characteristic of sinapine with an absorbance maximum of 485 nm as shown in Figure 1. The recovery of sinapine following the extraction process was 100%

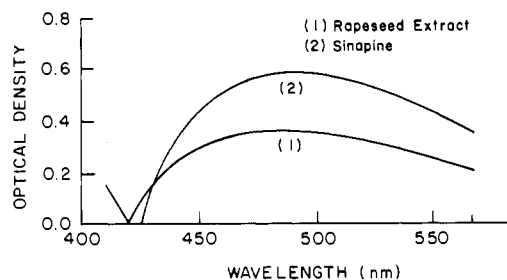


Figure 1. Absorbance spectra of titanium complexes with sinapine and rapeseed extract.

which confirmed no losses occurred during the extraction procedure. Unlike the titanium reagent the Reinecke salt can be added directly to the final extract. However, the time required for preparing the Reinecke salt, adjusting the dilution of the extract, plus the hour needed for the Reinecke salt-sinapine complex to form followed by centrifugation and solubilization of the complex in slightly acidified methanol is a far lengthier procedure than the 1-h period to dry the aliquot prior to the addition of $TiCl_4$. This is necessary as the reaction between sinapine and $TiCl_4$ proceeds best in concentrated HCl. This is distinct from the phenolic compounds where the reaction pro-

ceeded best in acetone. This study establishes the titanium method as a new and reliable procedure for the determination of sinapine. Studies are currently underway to establish the nature of the complex formed.

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A New Procedure for Extraction and Determination of Biphenyl in Citrus Fruit

Biphenyl is used extensively to protect overseas shipments of fresh citrus fruits. Low residue tolerances imposed by some importing countries have necessitated further research to insure protection by the fungicide without exceeding allowable levels. Biphenyl is commonly extracted by steam distillation, which requires 1-3 h. A method of direct solvent extraction, requiring only a few minutes, is described herein. This method consists of blending peel samples with ethyl acetate, filtering the extract, and analyzing aliquots of the extract by gas chromatography.

The fungistatic agent biphenyl has been used to prevent decay of fresh citrus shipments for many years. One or more kraft paper pads impregnated with biphenyl are enclosed in each box of fruit. The amount of biphenyl absorbed into the oils of the peel depends on variety, duration of exposure, temperature, pad placement, amount of biphenyl per pad, and fruit maturity (Norman et al., 1969, 1971; Rygg et al., 1964; Rajzman, 1965). Low tolerance levels have been imposed by importers, such as Japan and Belgium (70 ppm), but in a few instances the amounts of adsorbed biphenyl have exceeded those levels (Wardowski et al., 1979). Therefore, further research is needed to determine more precisely application procedures and residue levels so that the fungicide insures protection without exceeding tolerance limits.

As noted by Beernaert (1973), many papers have been published on the analysis of biphenyl, and in almost all instances, steam distillation was used for isolation of the material. One exception was ether extraction by Soxhlet of peel that had been air-dried 12 h, but no data showing recovery of biphenyl from fresh fruit were given (McCarthy and Winefordner, 1965). An automated procedure was proposed by Gunther and Ott (1966) based on a steam distillation method previously described (Gunther et al., 1963). In Japan, the present method of analysis involves a distillation procedure similar to that described by Newhall et al. (1954) and the collection of the distillate in a solvent such as cyclohexane as used by Hayashi et al.

(1972). We considered this procedure, which is also recommended by the Pharmaceutical Society of Japan (1971) and by the AOAC (1970), rather time consuming. To expedite our research, we developed the direct solvent extraction procedure described herein.

MATERIALS AND METHODS

Grapefruit from simulated shipping experiments were used as test material. Results of various treatments relating to factors affecting the rate of adsorption of biphenyl are presented elsewhere (Wardowski et al., 1979). Rinds of five fruit were cut into six equal sections, and one section of rind from each fruit was combined for analysis. The sections were cut into smaller pieces, placed in a blender (Waring explosion-proof), and ground 4 min with 300 mL of reagent grade ethyl acetate. The slurry was filtered with cheesecloth, and a small portion was centrifuged at 2000 rpm for 5 min. A 2- μ L portion of the supernatant was injected without further cleanup into a gas chromatograph. The instrument used was a Microtek GC-2000R with a 15% silicone gum rubber column, 80/100 mesh, SE-30 (methyl), 3 m \times 6 mm. Temperatures were as follows: oven, 175 $^{\circ}$ C; inlet, 200 $^{\circ}$ C; and flame ionization detector, 250 $^{\circ}$ C. The carrier gas was N_2 at 100 mL/min. Concentrations were determined by measurement of peak heights. For calculations, no adjustment of volume due to presence of water in the peel was considered necessary. Analysis by liquid chromatography (Davis and Munroe,