

DETERMINATION OF PHENOLIC ACIDS IN RAPESEEDS  
WITH THE HPLC METHOD

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Sinapic acid dominates over all phenolic acids occurring in rapeseeds. It is present in two isomeric forms: cis and trans /1,2,3,4/. Apart from these two forms of sinapic acid, methyl ester of sinapic acid is probably also present /5/. Disagreements as to the forms of sinapic acid present in rapeseeds induced the authors to undertake studies taking advantage of the HPLC method. Compared with other methods, and especially with GLC, HPLC methods makes it possible to avoid transformations of phenolic compounds during sample preparation/for instance, sililation/.

#### METHODS

Phenolic acids were extracted from rapeseeds according to the procedure presented in Fig. 1.

Phenolic acids were determined in Varian 8500 apparatus, using a column 250 mm x 4,6 mm filled with Lichrosord RP 18. Elution of phenolic acids from the column was carried out with a mixture of water, acetonitrile and acetic acid, at a ratio of 88: 10: 2. Analysis of phenolic acids was performed on 20 x 20 plates covered with cellulose MN 300. Plates were developed in 5% CH<sub>3</sub>COOH.

#### RESULTS

Analysis of the compounds extracted with 80% MeOH from several species and varieties of rapeseed with the TLC method confirmed the well-known fact that sinapine and sinapic acid represent main phenolic compounds in these seeds /Fig. 2/.

FIG.1 PROCEDURE OF ISOLATION OF FREE PHENOLIC ACIDS

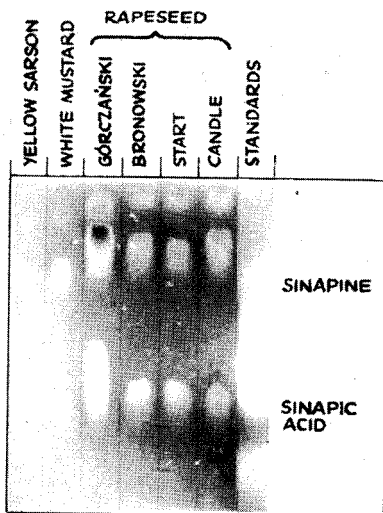
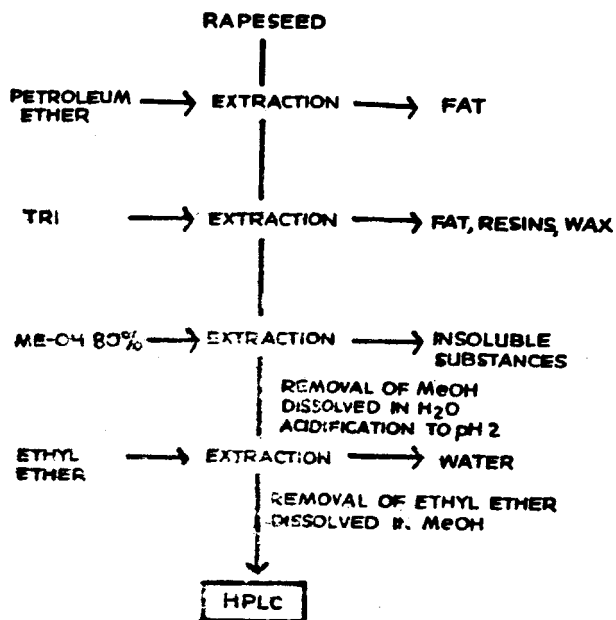
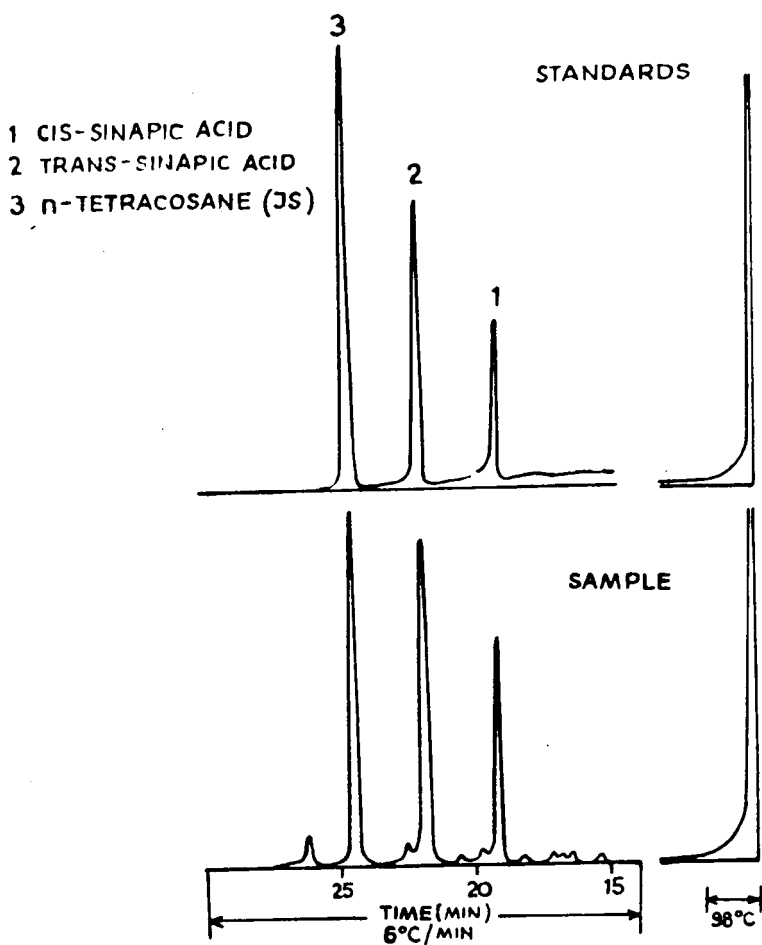


FIG.2 THE MAJOR PHENOLIC COMPOUNDS IN RAPESEED

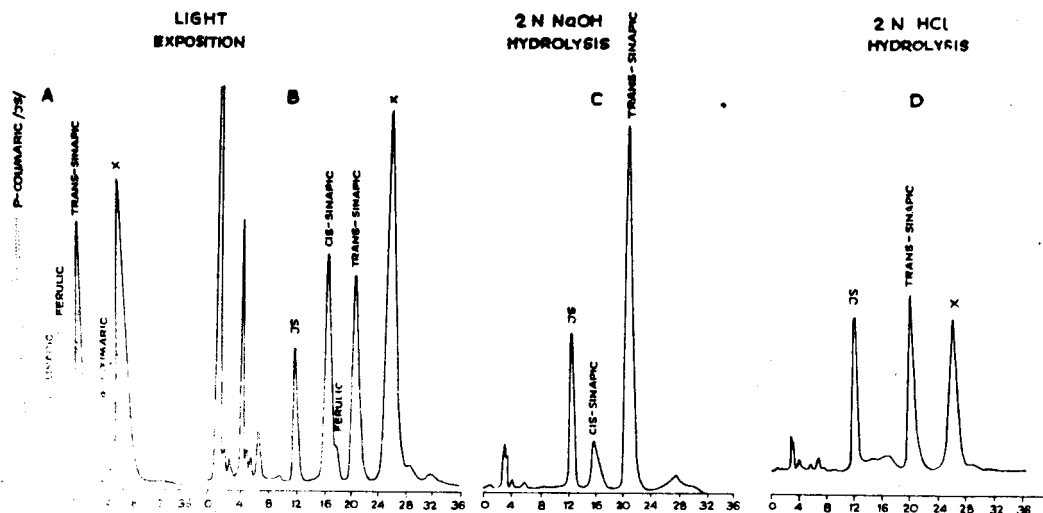
Analysis of free phenolic acids separated from rapeseeds of the Start variety taking advantage of GLC method revealed the presence of sinapic acid *cis* and *trans* /Fig. 3/.

FIG. 3 FREE PHENOLIC ACIDS /GLC/



Analysis of the same acids with HPLC method did not confirm the presence of cis sinapic acid. On the other hand, an X compound has been discovered, with retention time similar to that of o-coumaric acid /Fig/ 4 A/.

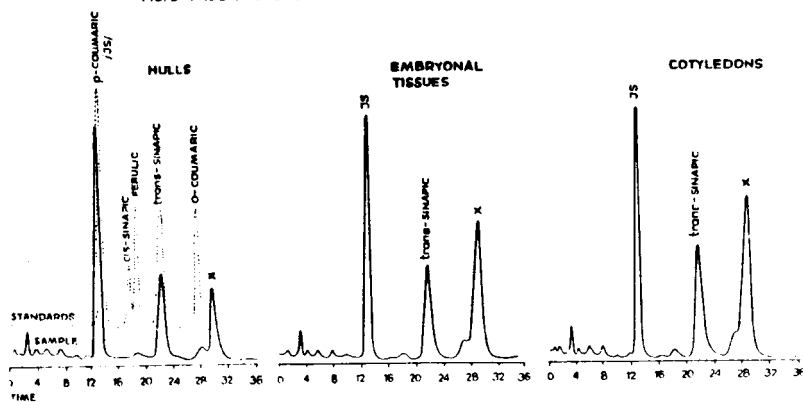
FIG. 4 FREE PHENOLIC ACIDS /HPLC/



It appeared that cis-form of sinapic acids is formed as a result of sample exposition /MeOH extract/ to light or of hydrolysis with 2 N NaOH /Fig. 4 B and C/. At the same time, it was observed that the compound X decomposed during alkaline hydrolysis, revealing the presence of o-coumaric acid and increasing the area of the peak of trans sinapic acid. It was established that this compound is only partly hydrolyzed under the effect of acid /Fig. 4 D/. Increase of the area of the peak of trans sinapic acid at the expense of X peak suggests a presence of sinapic acid derivative. Its structure is now under study.

Presence of an X compound and lack of cis-form of sinapic were noted all anatomic parts of rapeseeds, viz. hulls, embryonal tissue and cotyledons /Fig. 5/.

FIG. 5 FREE PHENOLIC ACIDS IN ANATOMIC PARTS OF RAPESEED /HPLC/



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