are also minute. Therefore, the hexane extraction step has been introduced for the purification of each phenolic fraction in the analytical scheme proposed in Figure 1. In general, the extraction and isolation procedures proposed require only one-fifth the time involved in previous methods (Kozlowska et al., 1975; Sabir et al., 1974). The procedure should greatly reduce the opportunity for formation of artifacts during the extraction and separation procedures (Fenton et al., 1980).

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Free, Esterified, and Insoluble-Bound Phenolic Acids. 2. Composition of Phenolic Acids in Rapeseed Flour and Hulls

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The composition of free, esterified, and insoluble-bound phenolic acids in rapeseed cultivars was determined by gas–liquid chromatography and mass spectrometry. Rapeseed hulls contained no free phenolic acids and relatively low levels of soluble ester and bound phenolics. Sinapic acid was the principal phenolic acid released by hydrolysis of the soluble esters in the hulls while protocatechuic acid was the major phenolic acid in the residues. The flours contained 6–98 mg/100 g of free phenolic acids, 768–1196 mg/100 g of phenolic acids from hydrolyzed esters, and no phenolic acids in the residues. Sinapic acid represented a high proportion of the free phenolic acids and 99% of acids released from esters in the flours. Minor phenolic acids included *p*-hydroxybenzoic, vanillic, gentisic, protocatechuic, syringic, *p*-coumaric, and ferulic acids in the various fractions and cultivars.

During the past decade, the quality of rapeseed has been improved markedly by plant breeding to reduce the levels of erucic acid in the oil and thioglucosides in the meal. Unfortunately, the "canola" type of rapeseed meal has limited feed applications due to its dark appearance, high proportion of hulls, and strong flavor. Yellow-seeded cultivars including the mustards are significantly lower in crude fiber content than black-seeded rapeseed (Sosulski, 1979). Recently a yellow/brown-seeded cultivar was developed in which hull and crude fiber levels are intermediate.

In addition to the reduction in meal fiber, plant breeders are seeking to improve the palatability of the canola-type meals by selecting for low levels of sinapine. Sinapine is the choline ester of sinapic acid which occurs in rapeseed meals at levels of 1.0–2.5% under Canadian conditions (Mueller et al., 1978). In addition to its adverse effects on meal flavor and palatability, sinapine is responsible for fishy odors in brown-shelled eggs when incorporated into poultry rations (Hobson-Frohock et al., 1975). Numerous other phenolic compounds have been identified in rapeseed meals and flours (Durkee and Thivierge, 1975; Kozlowska et al., 1975; Lo and Hill, 1972), but there is little quantitative information available on which to base a plant breeding and selection program.

The objectives of this investigation were to obtain qualitative and quantitative information on the phenolic acids in rapeseed by gas–liquid chromatography (GLC) and gas chromatography–mass spectrometry (GLC–MS) methods. Since many of the important phenolic acids and esters are highly reactive, the analyses were designed to separate the free, soluble ester, and insoluble-bound phenolic acids present in the flours and hulls based on the fractionation procedure of Krygier et al. (1982). The study was conducted on the defatted flours of yellow-, yellow/brown-, and black-seeded cultivars of rapeseed, as well as the hulls of the black cultivar.

EXPERIMENTAL SECTION

Materials. Analyses were conducted on freshly harvested seeds of the cultivars Yellow Sarson, strain R-500 (*Brassica campestris* L.), Candle (*B. campestris* L.), and Tower (*Brassicanapus* L.). The seeds were flaked, the hulls separated by hand, and meats ground and defatted

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Table I. Free Phenolic Acids in Defatted Flours (mg/100 g)

phenolic acid	Yellow Sarson	Candle	Tower
p-hydroxybenzoic	0.1	0.5	trace
vanillic	trace	0.3	0.8
gentisic		0.4	trace
protocatechuic	0.3	0.6	
syringic	1.1	0.6	1.5
p-coumaric		1.1	3.1
cis-ferulic		trace	0.6
trans-ferulic	0.8	6.8	2.7
caffeic	0.1	0.3	0.4
cis-sinapic	trace	0.7	9.0
trans-sinapic	3.7	73.2	80.1
chlorogenic		trace	trace
total	6.1	84.5	98.2

Table II. Characteristic Ions Obtained by Electron Ionization Mass Spectrometry of Me_3Si Derivatives of Malic, Sinapic, and Chlorogenic Acids in Rapeseed Flours and Their Relative Abundances in Percent (*m/e* 73 = 100)

malic acid		sinapic acid		chlorogenic acid	
m/e	abundance	*m/e*	abundance	*m/e*	abundance
423	0.7	368	93.6	786	2.8
335	7.4	353	38.1	372	3.0
245	14.8	338	87.0	345	47.5
233	31.7	323	17.0	307	27.5
189	13.6	279	21.4	255	36.6
175	8.9	249	19.4	219	11.4
147	71.2	169	8.6	191	10.7
133	11.9	162	21.9	147	19.5

twice with hexane. The hulls of the black-seeded Tower were also ground and defatted as for the flours.

Methods. The procedures for phenolic acid extraction, purification, hydrolysis, and quantitation have been previously described (Krygier et al., 1982). GLC–MS data were used to confirm the identification of phenolic acids and other compounds. The contents of phenolic acids are expressed as milligrams per 100 g of defatted flour or hulls.

RESULTS

As has been reported previously (Durkee and Thivierge, 1975), the hulls of Tower rapeseed showed no detectable levels of free phenolic acids. However, all flour samples contained *p*-hydroxybenzoic acid, vanillic, syringic, *trans*-ferulic, caffeic, and *cis*- and *trans*-sinapic acids (Table I). The total content of free phenolic acids was only 6.1 mg/100 g of flour in the Indian cultivar, Yellow Sarson. The Canadian cultivars, Candle and Tower, contained over 10 times this level, the difference being primarily due to the high levels of sinapic acid. In addition, the latter cultivars contained gentisic, *p*-coumaric, *cis*-ferulic, and chlorogenic acids which were not detected in Yellow Sarson.

A major peak which eluted early in the GLC chromatograms on 1.5% SE-30 ultraphase (Kozlowska et al., 1975) or on 3% OV-1 (Sosulski et al., 1980) was previously identified as *trans*-cinnamic or salicylic acids. Careful examination of the present chromatograms failed to confirm the presence of any phenolic compounds in this region. A major peak with a similar retention time (Figure 1) was identified by GLC–MS as malic acid (Table II).

Small quantities (trace = 2 mg/100 g) of eight phenolic acids appeared in the hydrolysate of the soluble esters extracted from Tower hulls (Table III). However 94.2% of the phenolic acids were *cis*- and *trans*-sinapic acids. Tower flour contained the same phenolic acids as the hulls in this fraction, both showing a significant level of *trans*-ferulic acid. However, the total esterified phenolic acids

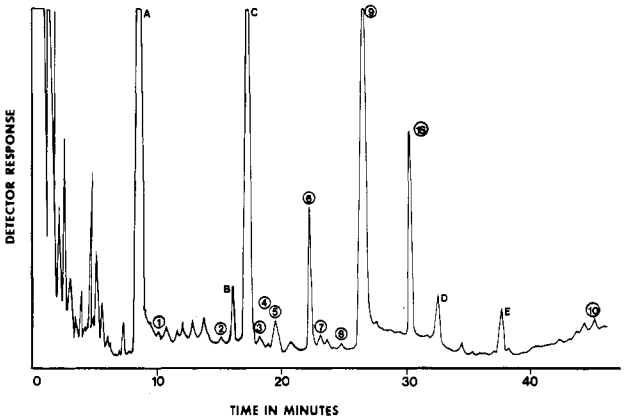

Figure 1. GLC chromatogram of Me_3Si derivatives of free phenolic acids in Tower flour [2.0 × 2 mm glass column packed with 3% OV-1 on 80–100-mesh Chromosorb W (HP)]. 1 = *p*-hydroxybenzoic, 2 = vanillic, 3 = syringic, 4 = *cis*-ferulic, 5 = *p*-coumaric, 6 = *cis*-sinapic, 7 = *trans*-ferulic, 8 = caffeic, 9 = *trans*-sinapic, and 10 = chlorogenic acids, A = malic acid, B = arabinofuranose, C = citric acid, and D and E = contaminants from plastics.

Table III. Phenolic Acids Liberated from Soluble Esters (mg/100 g of Flour)

phenolic acid	Tower hulls	Yellow Sarson flour	Candle flour	Tower flour
p-hydroxybenzoic	0.1	0.3	1.5	1.1
vanillic	0.5	trace	0.6	trace
protocatechuic	0.7		0.5	0.2
syringic	1.4		trace	trace
p-coumaric	0.6		trace	2.2
cis-ferulic	trace	trace		trace
trans-ferulic	1.9	5.9	5.5	8.7
caffeic	1.1			1.3
cis-sinapic	18.7	49.1	72.1	73.6
trans-sinapic	85.0	712.3	1116.2	894.9
total	110.0	767.6	1196.4	982.0
sinapic acids as % of total acids	94.3	99.2	99.3	98.6
sinapine as % of the flour	0.2	1.1	1.7	1.4

Table IV. Phenolic Acids Liberated from the Insoluble Residue (mg/100 g of Flour)

phenolic acid	Tower hulls
p-hydroxybenzoic	0.2
vanillic	1.2
protocatechuic	16.4
p-coumaric	trace
cis-ferulic	trace
trans-ferulic	trace
caffeic	4.3
trans-sinapic	2.4
total	24.5

in the flours were much greater than in the hulls, and 99% was the sinapic acid isomers. Candle flour contained the highest level of phenolic acids in this fraction (1196 mg/100 g) while Yellow Sarson contained primarily sinapic, ferulic, and *p*-hydroxybenzoic acids and a total of 768 mg/100 g.

The residues from the methanol/acetone extraction of the flours, after hydrolysis with 4 N NaOH, failed to yield any liberated phenolic acids. The GLC chromatograms showed a dozen or more major peaks, but GLC–MS analysis demonstrated that none were phenolic acids. Tower hulls were found to contain several insoluble-bound phenolic acids (Table IV). Protocatechuic acid represented two-thirds of the liberated phenolic acids; caffeic,

trans-sinapic, and vanillic acids were also present in the hulls.

The total contents of phenolic acids as a percentage of the flour were 0.8% for Yellow Sarson, 1.3% for Candle, and 1.1% for Tower, the hulls containing only 0.1%. Assuming all of the sinapic acid was present as sinapine, the contents of this choline ester would be 0.2% for hulls and 1.1., 1.7, and 1.4%, respectively, for Yellow Sarson, Candle, and Tower flours. It should be noted that Krygier et al. (1982) obtained sinapic acid recoveries of 63.5% by the present extraction procedures.

DISCUSSION

The presence of 10 free phenolic acids in rapeseed flours was confirmed by GLC–MS in the present study, although cultivar differences in composition were quite marked with only 6 mg/100 g in Yellow Sarson (Table I). These results are in contrast to those of Durkee and Thivierge (1975), who failed to isolate any free phenolic acids in rapeseed flour and hulls, or Fenton et al. (1980), who isolated only sinapic acid. Kozlowska et al. (1975) tentatively identified free *p*-hydroxybenzoic, *p*-coumaric, ferulic, caffeic, and chlorogenic acids in rapeseed flour but were unable to separate the vanillic, gentisic, protocatechuic, and syringic acid peaks, which was accomplished on the capillary columns used in this study. Lo and Hill (1972) also found caffeic and chlorogenic acids in rapeseed meals. The mass spectra data for the chlorogenic acid peaks detected in Candle and Tower flours are presented in Table II.

Durkee and Thivierge (1975) reported the presence of eight phenolic acids while Fenton et al. (1980) found six phenolic acids in base-hydrolyzed extracts of rapeseed meals. There is general agreement on the presence of *p*-hydroxybenzoic acid, vanillic, protocatechuic, ferulic, and sinapic acids in this fraction. Some cultivars in the present study also contained syringic, *p*-coumaric, and caffeic acids. Kozlowska et al. (1975) also found caffeic acid in hydrolysates of rapeseed extracts.

Sinapic acid, in the free and esterified forms, was the predominant phenolic acid in rapeseed flour. Besides the well-known *trans*-sinapic acid peak, a second sinapic acid like compound eluted approximately 4 min prior to the natural trans isomer (Figure 1). The mass spectra data of the Me_3Si derivatives of this compound and authentic *trans*-sinapic acid gave the same major fragments with essentially the same relative abundance of ions (Table II). Alkaline treatment of authentic *trans*-sinapic acid (4 N NaOH; 4 h; room temperature) was done to produce the cis and trans forms, and the mixture exhibited peaks with the same retention times as the sinapic acid isomers present in rapeseed samples. Further, the GLC–MS spectra of the cis and trans mixture were nearly identical with that of authentic *trans*-sinapic acid. There appeared to be no doubt that the two sinapic acids were the cis and trans isomers. Similar experiments were employed to confirm the presence of cis and trans isomers of ferulic acid, which ranked next to sinapic acid in importance in both free and esterified phenolics in the flours.

Apparently the cinnamic acids occur naturally in the more stable trans form but can be partially converted to the cis form by the action of light, especially UV (Ribereau-Gayon, 1972). The above alkali treatment converted about 2% of the *trans*-sinapic acid to the cis form in the present investigation. Isomerization was even noted during storage of silylated extracts in the refrigerator. According to Ribereau-Gayon (1972), derivatives of cinnamic acids such as chlorogenic acid occur naturally as cis and trans isomers. This would account for the higher ratio of cis to trans acids in the hydrolysates of the ester and glycoside fractions (Table III).

Sinapine is the principal phenolic acid ester in rapeseed; the concentrations are reported to vary from 0.6 to 1.2% (Austin and Wolff, 1968; Fenwick and Hoggan, 1976; Hobson-Frohock et al., 1975). The higher levels of 1.0–2.5% reported by Mueller et al. (1978) were expressed as sinapine bisulfate. In their study, sinapine represented 57–77% of the total sinapic acid esters, depending on the cultivar. Fenton et al. (1980) confirmed that sinapine was the major phenolic component but found at least seven other compounds that, on hydrolysis, yielded sinapic acid. The identity of other esters and glycosides, their relative proportions, and their influence on the flavor and color of flours should be determined before plant breeding or food utilization studies are undertaken.

Mueller et al. (1978) reported that *B. napus* cultivars were significantly higher in sinapine than *B. campestris* cultivars and that R-500 seed was lowest in sinapine content. In the present study, Yellow Sarson was also lower in free and esterified phenolic acids than Candle or Tower. However, Candle flour (*B. campestris*) was somewhat higher in total phenolic acids than Tower (*B. napus*), despite its lighter seed coat color. The low level of esterified and bound phenolic acids in Tower hulls indicates that hull characteristics have little influence on the content of simple phenolic compounds in seed or meal.

The limited variations in phenolic acids suggests that some gains could be made in the reduction of sinapine contents of cultivars by plant breeding. Selection would be simplified because over 98% of the total phenolic constituents are esters of sinapic acid.

The rapeseed flours appeared to contain no insoluble-bound phenolic acids, and the hulls contained primarily bound protocatechuic acid. Therefore, solvent extraction procedures would be effective in removing essentially all of the deleterious phenolic compounds from rapeseed, a technique which could be employed to produce a bland food-grade flour or protein concentrate.

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