Extraction of Phenolic Compounds from Canola during Protein Concentration and Isolation

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INTRODUCTION

The rapeseed and canola meals produced by commercial crushing plants are dark in appearance, fibrous and unpalatable for many feed as well as food applications. Even after dehulling, the refined flours exhibit adverse flavors and odors due to the high concentrations of glucosinolates and sinapine. The bitterness and astringency of canola flours are due primarily to the phenolic compound, sinapine, the choline ester of sinapic acid (Clandinin, 1961; Ismail et al., 1981). The concentrations of sinapine, glycosides of sinapic acid, and other phenolic compounds in rapeseed meals have been determined by numerous investigators (Durkee and Thivierge, 1975; Fenton et al., 1980; Krygier et al., 1982b; Muller et al., 1978). The characterization and quantitation of phenolic acids in canola meals and flours by fractionation into free, soluble ester and insoluble-bound constituents and quantitation using gas-liquid chromatography (GLC), aided by mass spectrometry, has been described by Sosulski et al. (1980), Krygier et al. (1982a) and Dabrowski and Sosulski (1983).

While analytical procedures have been developed for efficient extraction of phenolic compounds from rapeseed meals and flours, little consideration has been given to processes which might be applied on a commercial scale. Procedures have been described for the extraction of glucosinolates (Jones, 1979; Sosulski, 1978), phytate (Gillberg and Törnell, 1976; Siy and Talbot, 1982) and isolation of the protein from antinutritive factors (El Nockrashy et al., 1977). The objectives of the present investigation were to compare the various procedures for seed and flour extraction, protein concentration and isolation with respect to their efficiency in removal of phenolic compounds from canola flour.

MATERIALS AND METHODS

Four procedures for phenolic and protein extraction were applied to seeds of the canola cultivar, Candle, (Brassica campestris L.) as two stage extractions, solvent:solid ratio = 5:1, for 30 min each. Diffusion extraction of seed was conducted with dilute alkali (0.01N NaOH) at 80°C, followed by dehulling, drying and oil extraction with hexane (Sosulski, 1978). Aqueous extraction of meats was done at 30° and 80°C, followed by drying and oil extraction (Jones, 1977). Flour extractions were conducted with 70% ethanol at room temperature or with hot water, 80°C, after 15 min of boiling to denature proteins. Protein isolation involved

extraction with 0.02N NaOH, ratio 1:25, for 90 min, followed by centrifugation and isoelectric precipitation at pH 6.0, centrifugation, and then at pH 3.8 before freeze-drying the combined curds.

Free phenolic acids were extracted from canola flour, protein concentrates and isolate with tetrahydrofuran (Fig. 1) by the Dabrowski and Sosulski (1983) procedure. Methanol-acetone-water (7:7:6) was used to extract the phenolic esters and glycosides which were hydrolysed with 4N NaOH to release phenolic acids. The residues from the latter extraction were hydrolysed with 4N NaOH to release the insoluble-bound phenolic acids. The purified acids were derivatized with N, O-bis-(trimethylsilyl)-acetamide (BSA) and separated on a WCOT capillary column of fused silica (0.2 mm ID x 25 cm) coated with OV-1 in a Hewlett-Packard model 5880A chromatograph. The contents of phenolic acids are reported as the means of duplicate determinations in milligrams per 100 g of fat-free sample, dry basis (Fig. 2).

RESULTS AND DISCUSSION

Losses of flour solids during diffusion extraction of seeds removed 20% of the dry matter whereas other methods of protein concentration extracted 25-30% of the flour (Table 1). The yield of protein isolate was 37% of the original flour weight.

Protein contents of the concentrates at 54-61% and the isolate at 81% were low compared to commercial soybean products (Table 1). Ethanol reduced the lipid level to less than 1% while all extracted products were high in ash and fiber, except for fiber in protein isolate.

Table 1. Yield and proximate composition of canola flour, protein concentrates and isolate (%)

Extraction process	Yield, % of seed	Protein N x 6.25	Lipid %	Ash %	Fiber %
Control flour	36.5	48.2	1.1	7.5	8.8
Seed diffusion	29.2	59.0	1.8	6.6	10.1
Meats extraction	27.4	60.7	1.5	7.8	10.8
Flour ext.-alcohol	27.4	58.1	0.8	8.3	11.0
Flour ext.-water	25.2	54.2	2.8	8.0	11.2
Protein isolate	13.6	80.9	2.8	3.6	0.5

The untreated flour was devoid of free phenolic acids and hydrolysis of residues failed to indicate the presence of any bound acids (Table 2). However, the hydrolysis of soluble ester fraction yielded a high proportion, over 1.1% of the flour, of *trans*-sinapic acid, the *cis*-isomer being an artifact of the alkaline hydrolysis. *Trans*-ferulic and *p*-hydroxybenzoic acids were also found in significant quantities in the flour.

Free phenolic acids were found in all extracted products especially in the aqueous extracted flour (Table 2). Diffusion extraction of seed and ethanol washing of flour were the most effective processes in removing the esterified fraction, only 17-20% of the flour phenolics remained in this residue. Aqueous extractions of meats and flour left 34-40% of the phenolic esters in the extracted product while the protein isolate exhibited a similar concentration of phenolic acids from the ester fraction.

Table 2. Phenolic acid composition of canola flour, protein concentrates and isolate (mg/100 g of sample, dry basis)

Phenolic acids	Control flour	Seed diff. alkali	Meats extract. water	Flour extract. alcohol	Flour extract. water	Protein isolate alkali
Free acids						
trans-sinapic	-	1.4	3.2	2.0	6.9	2.1
Soluble esters						
p-OH benzoic	6.8	2.3	2.1	2.2	3.2	3.0
vanillic	0.7	-	-	-	tr.	-
protocatechuic	0.5	-	-	-	-	-
syringic	0.4	tr.	-	-	tr.	-
p-coumaric	0.6	tr.	tr.	-	-	-
cis-sinapic	45.4	12.0	37.7	13.2	25.1	31.7
trans-ferulic	15.1	8.0	10.0	13.0	14.1	2.9
caffeic	tr.	-	tr.	-	tr.	-
trans-sinapic	1131.1	183.1	356.7	210.6	428.4	332.5
Residues						
Total	1200.6	206.8	409.7	241.0	477.7	372.2
Relative %	100	17.2	34.1	20.1	39.8	31.0

The failure of any process to remove all of the phenolic compounds can be explained on the basis that only single solvents were used in each case, and only two extractions were made in the case of the protein concentrate procedures. Krygier et al. (1982a) found that no single solvent was effective in removal of all phenolic constituents of canola flour, and up to six extractions with methanol-acetone-water (meal to solvent ratio 1:20) was needed to ensure complete extraction. Ismail and Eskin (1979) used three 30-min extractions with acetone-water (3:2) at a meal to solvent ratio of 1:20 for quantitative extraction of sinapine from canola flour.

The appearance of the freeze-dried protein products reflected the processing techniques. Products obtained from diffusion extraction of seeds or meats, and alcohol extraction of the flour, were as white as the original defatted flour (Table 3). Aqueous extraction of the flour gave a grey product while the protein

isolate was dark brown in color.

Table 3. Hunterlab Color Difference values for canola flour, protein concentrates and isolate.

Extraction process	L	a	ba
Control flour	88.3	-3.7	16.4
Seed diffusion	84.9	-2.4	12.8
Meats extraction	88.2	-3.7	14.6
Flour extraction-alcohol	87.0	-2.7	14.7
Flour extraction-water	76.4	-3.1	22.7
Protein isolate	59.5	+0.3	19.1

[a]L=100(white), L=0(black); +a=red, -a=green; +b=yellow, -b=blue.

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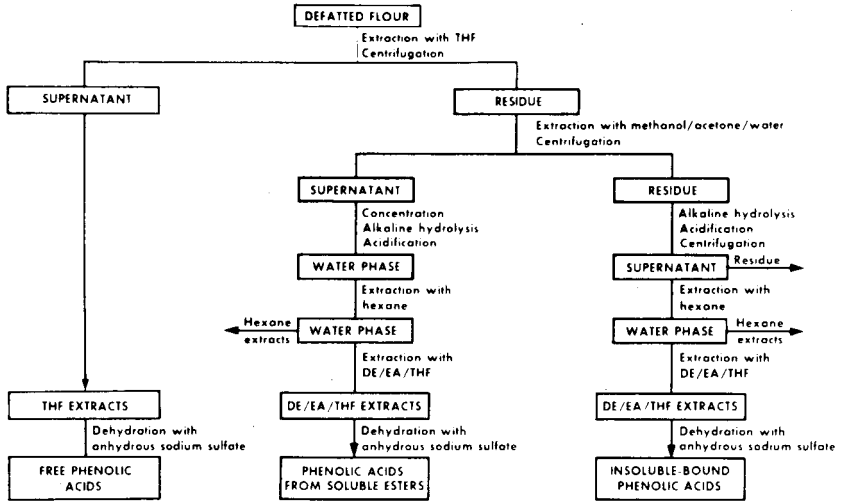

Fig. 1. Procedure for the extraction and separation of free, esterified and insoluble-bound phenolic compounds and their hydrolysis to phenolic acids for analysis by capillary GLC (DE/EA/THF = diethyl ether - ethyl acetate - tetrahydrofuran, 1:1:1).

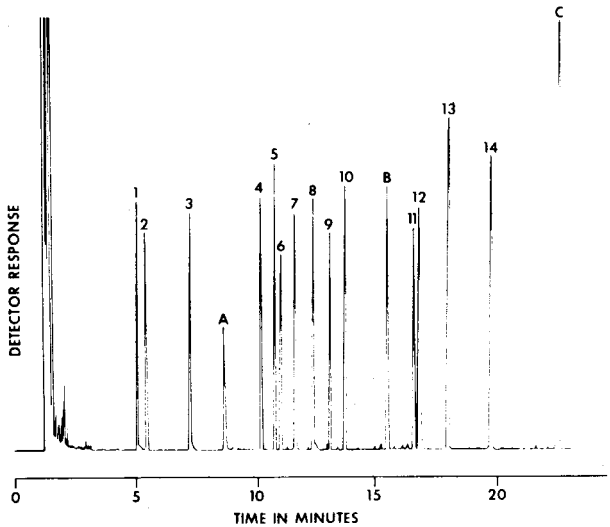

Fig. 2. GLC chromatogram of Me_3Si derivatives of reference phenolic acids on a WCOT capillary column of fused silica (0.2 mm i.d. x 25 m) coated with OV-101. 1=salicylic, 2=trans-cinnamic, 3=p-hydroxybenzoic, 4=vanillic, 5=gentisic, 6=o-coumaric, 7=protocatechuic, 8=m-coumaric, 9=syringic, 10=p-coumaric, 11=trans-isoferulic, 12=trans-ferulic, 13=caffeic, 14=trans-sinapic, A=butylated hydroxytoluene (preservative of tetrahydrofuran), B,C=internal standards, methyl heptadecanoate and n=tetracosane, respectively.