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The Effect of Methanol–Ammonia–Water Treatment on the Content of Phenolic Acids of Canola

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ABSTRACT

The contents of free, esterified and insoluble-bound phenolic acids of meals produced by methanol–ammonia–water/hexane treatment of canola seed were determined and compared with those of hexane-extracted meal. Ground seeds were extracted by the two-phase solvent extraction system consisting of 10% ammonia in methanol or 10% ammonia in methanol containing 5% water, and hexane as the second phase. The two-phase solvent extraction system removed 82% and 50% of the esterified and free phenolic acids originally present in the seed, respectively. However, the concentration of insoluble-bound phenolic acids was not affected.

INTRODUCTION

Canola varieties of rapeseed are low in both glucosinolates and erucic acid content. However, the content of their phenolic constituents is similar to the other varieties of rapeseed. Tentative identification of the principal phenolic compounds of rapeseed has been reported by Krygier *et al.* (1982*b*), and Kozlowska *et al.* (1975; 1983). Sinapic acid constituted over 73% of free phenolic acids and about 99% of phenolic acids released from esters and glucosides. Minor phenolic acids were *p*-hydroxybenzoic, vanillic, gentisic, protocatechuic, syringic, *p*-coumaric, ferulic and caffeic acids. In addition,

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trace amounts of chlorogenic acid was found in the free phenolic acids fraction. Choline ester of sinapic acid (sinapine) was found to be the major phenolic ester in rapeseed of Echo and Midas varieties. At least seven other compounds that on hydrolysis yielded sinapic acid (Fenton *et al.*, 1980) were also identified.

Phenolic compounds may contribute to the dark colour and bitter flavour of rapeseed meal. Therefore they are important factors when considering rapeseed meal as a protein source for food formulations (Kozlowska *et al.*, 1975). It has been reported that the treatment of oilseed meals by ammonia (gaseous or in alkanol solution) may reduce the content of the phenolics in treated meals. McGregor *et al.* (1983) found that gaseous ammoniation of *Brassica juncea* meal reduced the sinapine content by up to 74%. A much higher drop (up to 90%) in the phenolics content was reported by Kirk *et al.* (1966) for Crambe meal treated with gaseous ammonia. Extraction of cv. Candle and cv. Tower meals by ethanol containing 0·2M ammonia (Goh *et al.*, 1983) removed up to 82% and 39% of phenolics, respectively. These authors did not offer any explanation for the observed differences in the effectiveness of the treatment in the two seed varieties.

Methanol–ammonia treatment of canola seed reduced the total content of phenolic compounds by an average of 72·4% (Naczk *et al.*, 1986). Concentrations of ammonia in the methanol phase above 4% (w/w) did not affect the removal of phenolics from canola meals (Diosady *et al.*, 1985).

In this paper, we report the effect of the methanol–ammonia process on the content of free phenolics, esters of phenolic acids and phenolic acids structurally bound to proteins in canola varieties.

MATERIAL AND METHODS

Hexane-extracted canola meals were prepared by grinding the seeds in a Phillips coffee grinder and extracting for 12 h, using a Soxhlet apparatus. The defatted meal was dried at 40°C in a vacuum oven.

The methanol–ammonia solutions were prepared by bubbling anhydrous ammonia through methanol as such or methanol containing 5% (v/v) water at 0°C. The concentration of dissolved ammonia was determined by titration with 1·0N H_2SO_4. The 10% content of ammonia in absolute or 95% methanol was made up by dilution with ammonia-free solvent.

Ground seed (60 g) was blended at approximately 15 000 rpm in a Waring blender for 2 min with 400 ml 10% (w/w) ammonia in methanol or with 400 ml 10% (w/w) ammonia in methanol containing 5% (v/v) water. After a quiescent period of 15 min, 400 ml of hexane was added and the mixture was again blended for 2 min. The meal was separated by vacuum filtration,

rinsed three times with 100 ml methanol and dried at 40°C in a vacuum oven. The residual oil was further extracted with hexane using a Soxhlet apparatus and the resultant meal was dried again as before.

The free phenolics, soluble esters of phenolic and insoluble-bound phenolic acids were isolated using the procedure of Krygier *et al.* (1982*a*). Meals (2 g) were extracted six times with a 40 ml mixture of methanol–acetone–water (7:7:6) at room temperature, using a Polytron (Brinkman) (15 s, 10 000 rpm). After each centrifugation (15 min, 5000 rpm), the supernatants were collected and combined, evaporated at 30°C under vacuum to approximately 40 ml, and extracted six times with diethyl ether at a supernatant-to-solvent ratio of 1:1. The ether extracts were combined and evaporated to dryness at 30°C under vacuum. The extracted phenolic acids (referred to as free phenolic acids) were dissolved in methanol. The supernatant containing esterified phenolic acids was then treated with 30 ml 4N NaOH under nitrogen for 4 h at room temperature. The resultant hydrolyzate was acidified to pH = 2 using 6N HCl, and extracted six times with diethyl ether. The ether extracts were combined and evaporated to dryness at 30°C under vacuum. The extract of phenolic acids liberated from their esters was dissolved in methanol. The left over meal after extractions was treated with 20 ml 4N NaOH under nitrogen for 4 h at room temperature. The mixture was then acidified with 6N HCl to pH = 2 and centrifuged (15 min, 5000 rpm). The supernatant was extracted six times with diethyl ether. The ether extracts were combined and evaporated to dryness at 30°C under vacuum. The acids liberated from insoluble residue (referred to as insoluble-bound phenolic acids) were dissolved in methanol. The contents of phenolic acids in methanol were determined colorimetrically by the method of Swain & Hillis (1959) as follows. To 0·5 ml methanol solution of phenolics, 0·5 ml Folin-Denis solution, 1 ml sodium carbonate-saturated solution and 8 ml water were added and mixed well. Absorbance was measured at 725 nm, after 30 min standing at room temperature; *trans*-sinapic acid was used as standard in these experiments. The contents of free phenolics, soluble esters of phenolic acids, and phenolic acids liberated from insoluble residues were expressed as *trans*-sinapic acid equivalents (mg/100 g of meal, on dry basis) using the equation $c = k\ (0{\cdot}174 \times A_{725} - 0{\cdot}012)$, correlation coefficient $r = 0{\cdot}998$, where k is a constant.

RESULTS AND DISCUSSION

Phenolic compounds in rapeseed are in free, insoluble-bound forms and as soluble esters and glycosides. About 80% of the total phenolic compounds, expressed as *trans*-sinapic acid equivalents, in canola meals were liberated

from the soluble esterified phenolic acids (Table 1). The content of soluble phenolic esters of hexane-extracted canola meals was 1202 to 1470 mg per 100 g of meal, depending on the variety of canola meal used. The meals also contained 244 to 268 mg of free phenolics and 96 to 101 mg of insoluble-bound phenolics per 100 g of meal. These results are in reasonable agreement with those reported by Krygier *et al.* (1982*b*) for dehulled Candle and Tower flours.

TABLE 1
Effect of Methanol–Ammonia Treatment on the Content of Phenolic Acids of Canola[a]

Meal	*Phenolic acids (mg trans-sinapic acid equivalent/100 g meal (on dry basis))*			
	Free	*Soluble esters*	*Insoluble-bound*	*Total*
Tower				
Hexane-extracted	244 ± 4	1 202 ± 42	96 ± 2	1 542 ± 43
Extracted with 10% ammonia in methanol and hexane	128 ± 2	267 ± 2	76 ± 1	471 ± 3
Extracted with 10% ammonia in methanol containing 5% water and hexane	105 ± 2	202 ± 2	76 ± 1	383 ± 3
Regent				
Hexane-extracted	262 ± 8	1 470 ± 24	105 ± 3	1 837 ± 26
Extracted with 10% ammonia in methanol and hexane	122 ± 1	193 ± 9	87 ± 2	402 ± 10
Extracted with 10% ammonia in methanol containing 5% water and hexane	135 ± 2	228 ± 9	103 ± 2	466 ± 4
Altex				
Hexane-extracted	248 ± 4	1 458 ± 31	101 ± 2	1 807 ± 32
Extracted with 10% ammonia in methanol and hexane	143 ± 2	267 ± 6	112 ± 2	522 ± 7
Extracted with 10% ammonia in methanol containing 5% water and hexane	137 ± 3	253 ± 7	115 ± 1	505 ± 8

[a] Results are mean values of at least five replicates ± standard deviation.

The methanol–ammonia/hexane and methanol–ammonia–water/hexane process removed the esterified phenolic acids more effectively than the free phenolics (Table 1). However, no significant ($p = 0{\cdot}05$, t-test) effect was observed on the content of insoluble-bound phenolic acids in the resultant meal. Preferred extraction of esterified phenolic acids by methanol–ammonia and methanol–ammonia–water solutions brought about changes in the distribution of phenolic compounds in the treated meal as compared to those in the hexane-extracted meal (Fig. 1). The extraction of ground canola seeds with methanol–ammonia removed 77·8 to 86·9% (average 82·1%) of the esterified phenolic acids and about 50% of free phenolic acids originally present in the seeds. Finally, between 69·4 and 78·1% of the total phenolics present in seeds were removed by this process, depending on the variety of canola used. Furthermore, presence of 5% (v/v) water in methanol–ammonia solution had little influence on the efficiency of the removal of phenolic compounds from canola seeds.

The meals obtained from the methanol–ammonia/hexane or methanol–ammonia–water/hexane extraction process had a sandy, light-beige colour

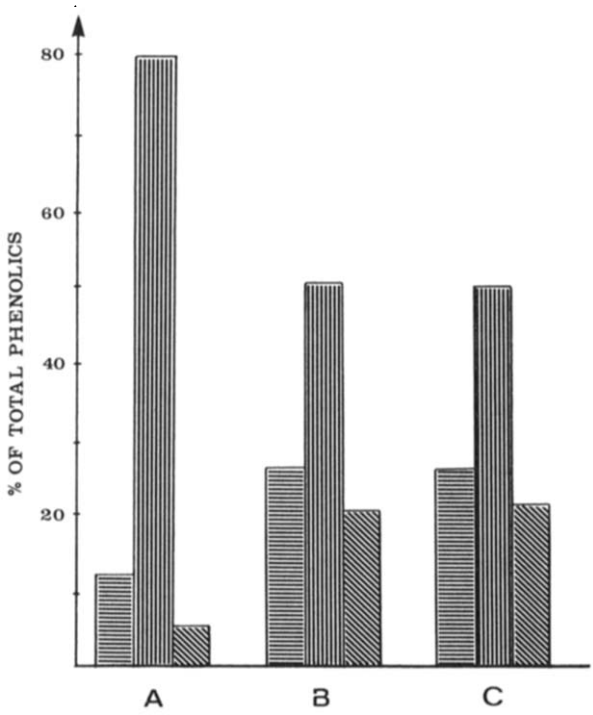

Fig. 1. Effect of processing on the distribution of free ( ), esterified ( ) and insoluble-bound phenolic acids ( ) in Altex meals: A, Hexane extracted; B, extracted with 10% (w/w) ammonia in methanol and hexane; C, extracted with 10% ammonia in methanol containing 5% (v/v) water and hexane.

and were bland in taste. Thus, the two-phase solvent extraction system has a beneficial effect on the quality of the meal produced.

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