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Recovery of rapeseed tannins by various solvent systems

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Recovery of tannins from commercial canola meals as affected by various solvent extraction systems and conditions was investigated. Solvents tested were methanol, acetone, *N,N*-dimethylformamide, as well as their combinations with water or concentrated HCl. Pure solvents were inefficient extraction media for the recovery of phenolics and particularly tannins. However, addition of water, up to 30% (v/v), greatly improved their effectiveness for extraction of tannins; 70% acetone or 70% *N,N*-dimethylformamide were the most effective solvent extraction systems for the recovery of rapeseed tannins. A two-stage extraction of meal with 70% solvents was sufficient for total extraction of soluble tannins. Addition of concentrated HCl to the extraction medium lowered the recovery of tannins from commercial canola meal.

INTRODUCTION

Phenolic compounds may contribute to the dark colour, bitter taste and astringency of rapeseed meals. They and/or their oxidized products may also form complexes with essential amino acids, enzymes and other proteins, thus lowering the nutritional value of rapeseed products. Therefore, phenolic compounds are important factors influencing the nutritional value of rapeseed meal as a protein source in food/feed formulations. However, little information on the undesirable effects of phenolics on the quality of rapeseed meals is available (Kozlowska *et al.*, 1975; Sosulski, 1979).

Tannins are complex polyphenolic compounds present in a wide variety of foods and feeds of plant origin. They occur in the hydrolyzable or condensed form. Condensed tannins are formed by polymerization of flavan-3-ols and flavan-3,4-diols, while hydrolyzable tannins belong to gallotannins or ellagitannins. Condensed tannins may form complexes with proteins, thus lowering the nutritional value of products. Presence of condensed tannins in rapeseed hulls was first reported by Bate-Smith and Ribereau-Gayon (1959). This finding was verified by Durkee (1971), who identified cyanidin, pelargonidin and, as an artefact, the *n*-butyl derivative of cyanidin, among the hydrolytic products of rapeseed hulls. According to Clandinin and Heard (1968), rapeseed meal contained approximately 3% tannins as assayed by the method of tannin determination in cloves and allspice (AOAC, 1965). However, Fenwick and Hoggan (1976) reported that these values included sinapine. Thus, their tannin content should be corrected for the presence of sinapine. In later work, Fenwick *et al.* (1984) demonstrated that whole and dehulled Tower meals contained 2·71 and 3·91% tannins, respectively. On the other hand, Blair and Reichert (1984) reported the presence of 0·09–0·39% tannins in the defatted rapeseed cotyledons and 0·23–0·54% tannins in the defatted canola cotyledons, as assayed by a modified vanillin method (Price *et al.*, 1978). Recently, Shahidi and Naczk (1988, 1989*a*,*b*) reported that canola varieties contained from 682 to 772 mg condensed tannins per 100 g of oil-free meal as assayed by the modified vanillin method. Only 556 and 426 mg tannins were present in high glucosinolate rapeseed varieties of Midas and Chinese cultivars

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of Hu You 9, respectively. The variability in the reported results in tannin content in rapeseed/canola is due to the differences in the solvent extraction systems employed for the recovery of tannins as well as the methods used for their quantification.

The objective of this study was to evaluate the effects of various solvent extraction systems as well as selected extraction conditions on the recovery of rapeseed/canola tannins.

MATERIALS AND METHODS

Commercial canola meal was extracted for 12 h using a Soxhlet apparatus and then dried at 50°C in a forced air oven for 18 h. The polyphenolics of rapeseed were isolated as follows. A 1 g sample of meal was extracted twice with 10 ml of methanol, acetone of *N,N*-dimethylformamide containing up to 50% (v/v) of water using a Polytron (PT 3000, Brinkman) (1 min, 10 000 rpm) at room temperature. After each centrifugation (10 min, 5 000 rpm), the supernatants were collected, combined and evaporated to dryness at 30°C under vacuum. The extracted tannins were dissolved in 10 ml methanol. The extraction conditions were varied for methanol, *N,N*-dimethylformamide and acetone as follows:

(i) water content in solvent: 0, 10, 20, 30 and 50% (v/v);
(ii) ratio of meal to solvent (*R*) containing 30% water, where *R* = 1 : 5, 1 : 10 and 1 : 20;
(iii) number of extraction steps: 1, 2, 4 and 6 for a solvent containing 30% water;
(iv) addition of 1% concentrated HCl to aqueous solvents with or without additional boiling;
(v) Solvent combinations.

The methanolic solution of polyphenolics was used for determinations of condensed tannins (vanillin-sensitive polyphenols) and total content of phenolics.

The condensed tannins were assayed colorimetrically by using the following modified vanillin method of Price *et al.* (1978): to 1 ml of methanolic solution of condensed tannins, 5 ml of 0·5% vanillin reagent (sample) or 5 ml of 4% concentrated HCl in methanol was added and mixed well. The absorbances of sample and blank were measured at 500 nm after a 20 min standing at room temperature. Catechin (+) (3·5 moles of water per mole of catechin, Sigma Chemical Co.) was used as a standard in these experiments. The content of tannins in each meal was expressed as catechin equivalents (mg per 100 g of meal, on dry weight basis) using the equation: $C = k^* [1{\cdot}6835 * A_{500} - 0{\cdot}039]$, correlation $r = 0{\cdot}999$, where k is a constant and C is the per cent content of tannins calculated as catechin equivalents in oil-free canola meal.

The total content of phenolics in methanolic solution was determined colorimetrically according to the method of Swain and Hillis (1959). To 0·5 ml of methanolic solution of phenolics, 0·5 ml Folin-Denis reagent, 1 ml saturated solution of sodium carbonate 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 a standard in these experiments. The per cent content of phenolics was expressed as *trans*-sinapic acid equivalents, on a dry basis, using the equation $C = k^* [0{\cdot}173 * A_{725} - 0{\cdot}012]$, correlation coefficient $r = 0{\cdot}991$, where k is a constant.

All assays were conducted at room temperature (about 22°C) using appropriate samples and blanks. Results presented in Tables 1–5 (see below) are mean values of at least six determinations. The maximum error (calculated as percentage of standard deviation/mean value) did not exceed 5%.

RESULTS AND DISCUSSION

In previous work, the authors showed that the recovery and quantification of tannins in rapeseed/canola meal were affected by the solvent extraction system employed (Shahidi & Naczk, 1989*a*,*b*). Therefore, the effectiveness of tannin recovery, as affected by water

Table 1. Effect of water content in the extraction solvent on the recovery of total phenolics and tannins (%)[a]

Water content (%, v/v)	Acetone		Methanol		*N,N*-Dimethylformamide	
	Total phenolics	Tannins	Total phenolics	Tannins	Total phenolics	Tannins
0	0·07	0·00	0·40	0·04	0·55	0·00
10	0·69	0·16	0·61	0·09	0·73	0·24
20	0·77	0·32	0·65	0·19	0·96	0·33
30	0·81	0·32	0·87	0·24	1·06	0·32
50	0·81	0·26	0·88	0·24	1·10	0·32

[a] Total phenolics and tannin content are expressed as trans-sinapic acid and catechin equivalents, respectively.

Table 2. Effect of solvent combinations on the recovery of tannins[a]

Solvent system (*R*)	Total phenolics (%)	Tannins (%)
MeOH : Me_2CO : H_2O (7 : 7 : 6)	1·00	0·22
MeOH : DMF : H_2O (7 : 7 : 6)	1·08	0·30
Me_2CO : DMF : H_2O (7 : 7 : 6)	1·11	0·31

[a] MeOH, Methanol; DMF, *N,N*-dimethylformamide; Me_2CO, acetone. *R*, volume ratio of solvent in the system. Total phenolics and tannin contents are expressed as trans-sinapic acid and catechin equivalents, respectively.

content, addition of concentrated HCl, meal to solvent ratio, combination of solvent systems and number of extraction steps used, was examined. The vanillin method for determination of the recovery of tannins was selected due to its specificity for flavanols and dihydrochalcones, both of which have a single bond at the 2,3-position of the pyran ring and free OH groups at positions 5 and 7 of the benzene ring (Sarkar & Horwarth, 1976).

Absolute methanol, methanol containing 1% concentrated HCl and 70% acetone, are the common solvent systems used for the recovery of plant tannins. Gupta and Haslam (1980) tried different solvent systems such as water, methanol, ethanol, propanol, acetone and dimethylformamide for the extraction of polyphenolics from sorghum grain. They found that methanol was the most useful solvent system. On the other hand, Maxon and Rooney (1972) as well as Price *et al.* (1978) suggested that sorghum tannins are best extracted with 1% concentrated HCl in methanol. Leung *et al.* (1979) selected 70% acetone for the extraction of tannins from rapeseed hulls. However, no data on the effectiveness of tannin recovery, as affected by addition of water to solvents of choice, are available. Table 1 shows the effect of addition of water, in acetone, methanol and *N,N*-dimethylformamide, on the extraction efficiency of rapeseed tannins. Results indicate that pure solvents were poor extraction media for the recovery of phenolics and particularly tannins. Additions of water, up to 30% (v/v), improved the effectiveness of tannin recovery from commercial rapeseed meals. 70% acetone and 70% *N,N*-dimethylformamide were more efficient for the recovery of tannins as compared to 70% methanol. Use of a higher proportion of water (>30%) did not enhance the recovery of tannins any further; however, it improved the extraction of other phenolic compounds. Also, combinations of solvents had some influence on the recovery of rapeseed tannins (Table 2). These data also indicate that rapeseed tannins differ in their solubility as compared to those found in sorghum and beans (Gupta & Haslam, 1980; Deshpande & Cheryan, 1987). The effect of number of extraction stages used for the recovery of tannins was also investigated. Results presented in Table 3 indicate that a two-stage extraction of meal with 70% acetone or 70% dimethylformamide was sufficient for the extraction of soluble tannins. Further extraction (up to six stages) improved only the effectiveness of extraction of other phenolic compounds. In one experiment, rapeseed tannins were

Table 3. Effect of number of extraction steps on the recovery of total phenolics and tannins[a]

No. of extraction steps	70% Acetone		70% Methanol		70% *N,N*-Dimethylformamide	
	Total phenolics	Tannins	Total phenolics	Tannins	Total phenolics	Tannins
1	0·72	0·27	0·84	0·13	0·86	0·26
2	0·81	0·32	0·87	0·24	1·06	0·32
4	0·97	0·33	1·03	0·24	1·13	0·32
6	1·08	0·33	1·08	0·24	1·13	0·30

[a] Total phenolics and tannin contents (%) are expressed as trans-sinapic acid and catechin equivalents, respectively.

Table 4. Effect of meal to solvent ratio (R) on the recovery of total phenolics and tannins[a]

R	70% Acetone		70% Methanol		70% *N,N*-Dimethylformamide	
	Total phenolics	Tannins	Total phenolics	Tannins	Total phenolics	Tannins
1 : 5	0·77	0·26	0·84	0·19	1·01	0·30
1 : 10	0·81	0·32	0·87	0·24	1·09	0·32
1 : 20	0·95	0·33	0·89	0·28	1·12	0·30

[a] Total phenolics and tannin contents (%) are expressed as trans-sinapic acid and catechin equivalents, respectively.

Table 5. Effect of addition of concentrated HCl and/or boiling on the recovery of total phenolics and tannins[a]

Solvent system	Total phenolics (%)	Tannins (%)
1% HCl in methanol	0·89	0·07
1% HCl in 70% methanol	1·08	0·23
1% HCl in 70% methanol + 4 min of boiling	1·05	0·11
1% HCl in 70% acetone	1·01	0·22
1% HCl in 70% acetone + 4 min of boiling	1·05	0·34

[a] Total phenolics and tannin contents are expressed as trans-sinapic acid and catechin equivalents, respectively.

extracted with 70% acetone by using a Polytron homogenizer (twice, 1 min, 10 000 rpm) and by using a shaker (twice, 10 min, 1000 rpm). The yield of extraction of rapeseed tannins by using a homogenizer was similar to that obtained by using a shaker.

The yield of tannin recovery was also influenced by the meal to solvent ratio (*R*) (Table 4). Changing *R* from 1 : 5 to 1 : 10 increased the extraction of tannins by 70% acetone from 0·26 to 0·32% and by 70% methanol from 0·19 to 0·24%. Further changes in *R* enhanced only the extraction of tannins by 70% methanol. Thus, a meal to solvent ratio of at least 1 : 10 should be used for an efficient extraction of tannins from canola meals. However, changing the speed of homogenization from 10 000 to 25 000 rpm did not affect the yield of tannins.

The effect of addition of concentrated HCl to 70% methanol or 70% acetone on the recovery of tannins is shown in Table 5. Results indicate that the presence of HCl in the extraction media lowered the recovery of tannins from commercial canola meal. However, boiling had a beneficial effect on the recovery of tannins by 70% acetone containing 1% concentrated HCl to a level similar to that found for this solvent without addition of HCl. These results are opposite to those reported by Price *et al.* (1978) who found that the addition of concentrated HCl to methanol improved the extraction of sorghum tannins, Thus, the present study suggests that the nature of rapeseed tannins as well as their solubility differs from those found in sorghum.

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