CRITICAL EVALUATION OF QUANTIFICATION METHODS OF RAPESEED TANNINS

by

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INTRODUCTION

The use of rapeseed meal in human nutrition has been considered for many years. However, this has not yet been achieved due to the presence of antinutritional factors such as phytic acid and phenolic compounds. The content of phenolics in rapeseed flour is much higher when compared to that found in flours obtained from other oleaginous seeds.

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

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 an artifact n-butyl derivative of cyanidin in the hydrolytic products of rapeseed hulls.

Clandinin and Heard (1968) reported that approximately 3% tannins were present in rapeseed meal as assayed by the method of tannin determination in cloves and allspice (AOAC, 1965). However, Fenwick and Hogan (1976) showed that this value included sinapine. Thus, the corrected concentration of tannins in rapeseed meals was 18% to 45% lower than those given by Clandinin and Heard (1968). In addition, Fenwick et al. (1984) demonstrated that whole and dehulled Tower meals contained 2.71% and 3.91% tannins. Leung et al. (1979) reported that rapeseed hulls contained only 0.1% acetone-extractable condensed tannins. They found that leucocyanidin was the basic unit of the isolated condensed tannins. Mitaru et al. (1982) reported that hulls from rapeseed and canola seed contained from 0.02% to 0.22% extractable tannins. 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. Recently, Shahidi and Naczk (1988, 1989a,b) reported that canola varieties contained from 682 to 772 mg condensed tannins per 100g of defatted meal as assayed by the modified vanillin method. Only 556 mg and 426 mg

tannins were present in high glucosinolate rapeseed varieties of Midas and Hu You 9, respectively. The variability in the reported results on tannin content in rapeseed/canola is due to the differences in the solvent extraction system employed for the recovery of tannins and the methods used for their quantification.

The objective of this study was to evaluate the effect of solvent extraction systems and the extraction conditions on the recovery of rapeseed/canola tannins as well as to determine the suitability of the vanillin method for their determination.

MATERIALS AND METHODS

Commercial canola meal was extracted for 12 hr using a Soxhlet apparatus and then dried at 50°C in a forced-air convection oven for 18 hr. Hexane-extracted canola meals were prepared by grinding the seeds (60g) in a coffee grinder and extracting the oil from them for 12 hr using a Sohxlet apparatus. The defatted meals were dried as before.

The condensed tannins were isolated as follows. A 1.0 g sample of meal was extracted twice with 10 mL of methanol or acetone containing 30% (v/v) of water using a Polytron homogenizer (Brinkman) (60 sec, 10,000 rpm) at room temperature. After each centrifugation (10 min, 5,000 rpm), the supernatants were collected, combined and evaporated to dryness under vacuum at 30°C. The extracted tannins were dissolved in 10 mL methanol. The experimental conditions were varied for methanol 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, 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 water-solvent system with or without additional boiling.

Except where the effect of vanillin concentration was under study, the condensed tannins were assayed colourimetrically by the method of Price et al. (1978) as described below. 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. Absorbance of sample or blank was measured at λ = 500 nm, after a 20 min standing at room temperature. Catechin (+) (3.5 moles of water per mole of catechin, Sigma Chemical Co. St. Louis, Missouri) was used as a standard in these experiments. The content of tannins in the meal was expressed as catechin equivalents (mg per 100g of defatted meal, on dry basis) using the following equation: $C = k * [1.6835 * A_{500} - 0.039]$, correlation coefficient r = 0.999, where k is a constant and C is the content of tannins in mg catechins equivalents per 100g of defatted canola meal. In one experiment, the vanillin content in methanol containing 4% concentrated HCl (vanillin reagent) was varied as follows: 0.5, 1, 2, 4, and 8%.

The total content of phenolics in methanolic solution was determined colourimetrically accoding 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 sodium carbonate-saturated solution and 8 mL water was added and mixed well. Absorbance was measured at λ = 725 nm, after 30 min standing at room temperature; trans sinapic acid was used as a standard in these experiments. The content of phenolics was expressed as trans sinapic acid equivalents (mg per 100g of defatted meal, on dry basis) using the equation $C = k*[0.173*A_{725} - 0.012]$, correlation coefficient r = 0.991, where k is a constant.

RESULTS AND DISCUSSION

In a previous study we have shown that the recovery and determination of tannins in rapeseed/canola meal are affected by the solvent system used (Shahidi and Naczk, 1989). In the present work we used defatted commercial canola meal to study, in detail, the effectiveness of tannins recovery as affected by water content, addition of concentrated HCl, solvent to meal ratio, as well as number of extraction steps involved.

Absolute methanol, methanol containing 1% concentrated HCl and 70% acetone are the common solvent systems used for the recovery of plant tannins. Table 1 shows the effect of water in acetone and methanol on the extraction of rapeseed tannins. Results indicate that pure acetone and methanol to be very poor extraction media for the recovery phenolics and particularly tannins. Addition of water up to 30% (v/v) improves the effectiveness of tannins recovery from commercial meals. Use of 70% acetone was more efficient for the recovery of tannins as compared to 70% methanol. Use of a higher proportion of water (>30%) did not have any great effect on the recovery of tannins, however, it enhanced the extraction of other phenolic compounds.

The effect of the number of extraction stages for the recovery of tannins was also studied. Results (Table 2) indicate that a two-stage extraction of meal with 70% acetone or 70% methanol was sufficient for the extraction of tannins. Further extraction (up to six stages) improved only the effectiveness of extraction of other phenolic compounds.

The yield of tannin recovery was also influenced by the seed-to-solvent ratio (R) (Table 3). Changing R from 1:5 to 1:10 increased the extraction of tannins by 70% acetone from 257.3 to 321.3 mg per 100g of defatted meal. Thus, a seed-to-solvent ratio of at least 1:10 should be used for an efficient extraction of tannins from canola meals.

Different colourimetric methods are used for the determination and quantification of phenolic compounds including tannins. Price et al. (1978) and Desphande and Cheryan (1985) have critically re-evaluated the vanillin reaction for detection of tannins in sorghum and dry beans, respectively. In this study we re-examined the suitability of vanillin method for the evaluation of rapeseed and canola tannins.

Table 5 summarizes the absorption intensity of the tannin-vanillin complex during its formation. The colour intensity of tannin-vanillin complex at 500 nm increased during standing in dark for up to 10 min. Further standing for up to 30 min had little influence on further association/dissociation of the pigment. A longer standing period, however, resulted in slow dissociation of the complex and a decline in the aborbance at 500 mm was observed.

The absorption intensity of vanillin tannin complex depended only slightly on the concentration of vanillin reagent. The data presented in Table 6 indicate that increasing the concentration of vanillin reagent from 0.5% to 2.0% marginally increased the absorption intensity of vanillin-tannin complex. A further increase in the concentration of the reagent reversed this trend.

Table 1 Effect of water content in the extraction solvent on the recovery of total phenols and tannins

Water content (%; v/v)	Acetone		Methanol	
	Total phenols	Tannins	Total phenols	Tannins
0	66.0	0	402.3	35.1
10	687.3	156.3	609.6	87.3
20	770.7	321.3	653.7	190.0
30	805.8	321.3	874.0	241.8
50	810.4	260.7	879.5	234.9

Table 2 Effect of the number of extraction steps on the recovery of total phenols and tannins

Number of extraction steps	70% Acetone		70% Methanol	
	Total phenols	Tannins	Total phenols	Tannins
1	720.0	268.1	837.4	127.7
2	805.8	321.3	874.0	241.3
4	972.1	328.0	1025.7	243.8
6	1075.0	331.3	1081.0	235.5

Table 3 Effect of meal to solvent ratio (R) on the recovery of total phenols and tannins

R	70% Acetone		70% Methanol	
	Total phenols	Tannins	Total phenols	Tannins
1 : 5	773.5	257.3	844.3	188.3
1 : 10	805.8	321.3	874.0	241.3
1 : 20	948.0	324.6	886.7	275.4

Table 4 Effect of HCl addition and/or boiling on the recovery of total phenols and tannins

Solvent system	Total phenols	Tannins
1% HCl in Methanol	892.1	73.0
1% HCl in 70% Methanol	1079.8	225.9
1% HCl in 70% Methanol + 4 min. of boiling	1053.3	112.5
1% HCl in 70% Acetone	1010.7	216.9
1% HCl in 70% Acetone + 4 min. of boiling	1051.6	338.7

Table 5 Effect of standing time, in dark, on the absorbance of vanillin-tannin complex

Standing Time [min]	A_{500}
1	0.280
3	0.385
6	0.436
10	0.447
20	0.450
30	0.444
60	0.435

Table 6 Effect of vanillin concentration on the absorbance of vanillin-tannin complex

Vanillin Concentration [%]	A_{500}
0.5	0.188
1.0	0.203
2.0	0.204
4.0	0.196
8.0	0.189

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