An Overview of the Phenolics of Canola and Rapeseed: Chemical, Sensory and Nutritional Significance

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Utilization of rapeseed meal in human foods has been thwarted by the presence of antinutritional factors such as glucosinolates, phenolics, phytates and hulls. The content of phenolics in rapeseed flour is nearly 30 times higher than that of soybean. Phenolic compounds contribute to the dark color, bitter taste and astringency of rapeseed meals. They may also interact with amino acids, enzymes and other food components, thus influencing the nutritional value of rapeseed meal and its products. Therefore, phenolic compounds are important factors when considering rapeseed meal as a protein source in food formulations. Available literature data on phenolic compounds and tannins of rapeseed/canola are fragmentary and diverse. Furthermore, developing a standardized method for analysis and quantitation of these compounds is warranted. Interaction of rapeseed phenolics/tannins with proteins and their effects on enzymes and other food components remain to be studied.

KEY WORDS: Antinutrients, canola, color, nutrition, phenolic acids, rapeseed, sensory properties, tannins, taste.

Rapeseed is among the world's most important oilseed crops, and in Canada it is second only to wheat in value and area planted (1). Rapeseed is used for production of a high-quality edible oil and a feed-grade meal. Rapeseed meal has a reasonably well-balanced amino acid content (2) and a favorable protein efficiency ratio (3). However, utilization of rapeseed meal as a source of protein in human nutrition is limited by the presence of glucosinolates, phenolic compounds, phytates and hulls. The composition of rapeseed has been altered significantly by Canadian breeders who have developed the canola varieties, which contain less than 2% erucic acid in their oil and no more than 30 μmol aliphatic glucosinolates per gram of their defatted meal (4). In spite of introducing double-zero rapeseed varieties (canola) to common cultivation in many countries and patenting a number of methods for dehulling of rapeseed (5–8), use of rapeseed meal as a source of food-grade protein is still thwarted by the presence of phytic acid and phenolic compounds. The content of phenolics in rapeseed flour is much higher than in other oleaginous seeds and amounts to about 30 times that in soybean flour (Table 1).

Phenolic compounds may contribute to the dark color, bitter taste and astringency of rapeseed meals. They and/or their oxidized products also may form complexes with essential amino acids, enzymes and other proteins, thus lowering the nutritional value of the rapeseed product. Therefore, phenolic compounds are important factors when considering the nutritional value of rapeseed meal as a protein source in food formulations. However, the available information on the undesirable effects of rapeseed phenolics is still diverse and fragmentary (9,10). An overview of the available literature is provided and research needs in this area are described.

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TABLE 1

Total Content of Phenolic Acids in Some Oilseed Flours[a]

Flour	Total phenolics (mg/100 g)
Soybean	23.4
Cottonseed	56.7
Peanut	63.6
Rapeseed	639.9

[a]Reference 72.

PHENOLIC ACIDS

General considerations. The total content of phenolic acids in various rapeseed protein products ranges from 1542 to 1837 mg per 100 g defatted meal, on a dry weight basis (dwb), and from 623.5 to 1280.9 mg per 100 g of flour (dwb) (11–15). Phenolic acids of rapeseed are present in the free, esterified and insoluble-bound forms.

Free phenolic acids. Free phenolic acids contribute from 6.5 to 9.0% of the total phenolic acids present in most rapeseed flours (16) and up to 15% in canola meals (15) (Table 2). On the other hand, only trace amounts of free phenolics were found in flours obtained from Yellow Sarson variety (13). No detectable quantity of free phenolic acids was found in hulls of Tower rapeseed (17).

Data presented in Tables 2 and 3 indicate that sinapic acid {2[3-(4-hydroxy-3,5-dimethoxyphenyl)]propenoic acid} constituted 70.2–85.4% of the free phenolic acids of defatted meals (18). However, *p*-hydroxybenzoic, vanillic (4-hydroxy-3-methoxybenzoic), gentisic(2,5-dihydroxybenzoic), protocatechuic(3,4-dihydroxybenzoic), syringic(4-

TABLE 2

Contents of Free, Esterified and Insoluble-Bound Phenolic Acids in Some Rapeseed Products

Product[a]	Phenolic acids (mg/100 g)			
	Free	Esterified	Insoluble-bound	Total
Tower flour[b]	98.2	982	—	1080.2
Candle flour[b]	84.5	1196.4	—	1280.9
Tower meal[c]	244	1202	96	1542
Altex meal[c]	248	1458	101	1807
Midas meal[d]	144.5	1524	68.7	1736.2
Triton meal[d]	61.5	1212	51.3	1324.8
Mustard meal[d]	108.1	1538	22.4	1668.5

[a]Tower, Altex and Triton are *B. napus* canola, Candle is *B. campestris* canola, Midas is *B. napus* rapeseed and mustard is *B. juncea.*

[b]Reference 13. [c]Reference 15. [d]Reference 18.

TABLE 3

Content of Sinapic Acid in Different Fractions of Phenolic Constituents of *Brassica* Oilseed Meals[a]

Meal	Sinapic acid content in phenolics[b]		
	Free	Esterified	Insoluble-bound
Hexane extracted			
Midas	103.5	1081	5.1
Triton	43.2	1172	8.0
Mustard	92.3	1116	7.2
MeOH-NH_3-H_2O/ hexane extracted			
Midas	67.7	174.8	7.0
Triton	27.6	172.3	7.1
Mustard	24.5	161.3	13.9

[a]Reference 18.

[b]Expressed in mg sinapic acid per 100 g meal, on a dry basis.

hydroxy-3,5-dimethoxybenzoic), *p*-coumaric {3-(4-hydroxyphenyl)-2-propenoic}, *cis*- and *trans*-ferulic {3-(4-hydroxy-3-methoxyphenyl)-2-propenoic}, caffeic {3-(3,4-dihydroxyphenyl)-2-propenoic} and chlorogenic {3-[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]-1,4,5-trihydroxycyclohexane carboxylic} acids were present in small amounts (13).

Esterified phenolic acids. Esterified phenolic acids constituted up to 80% of phenolic acids present in rapeseed meals. However, canola flours contained from 91 to 93.5% of their phenolic acids in the esterified form (Table 2).

Rapeseed flours obtained from Polish rapeseed varieties (Start, Gorczanski and Bronowski) contained from 520 to 700 mg per 100 g meal of total phenolic acids liberated from esters (19). Canola meals obtained from Tower, Candle, Regent and Altex varieties contained up to 1458 mg per 100 g of phenolic acid liberated from esters (13,15) (Table 2). Tower hulls contained only 110.0 mg of soluble phenolic esters per 100 g sample (13).

From the data presented in Tables 2 and 3, sinapic acid constituted 70.9–96.7% of the soluble fraction of esterified phenolic acids in rapeseed meals (18). Small quantities of *p*-hydroxybenzoic, vanillic, protocatechuic, syringic, *p*-coumaric, *cis*- and *trans*-ferulic and caffeic acids also were present in the hydrolysates of the soluble esters extracted from Tower and Candle flours (13).

Sinapine, the choline ester of sinapic acid, and at least seven other compounds yielding sinapic acid upon hydrolysis were isolated from rapeseed meals of Midas and Echo varieties (20). A higher content of sinapine was found in *Brassica napus* rapeseed cultivars (1.65–2.26%) than in *Brassica campestris* cultivars (1.22–1.54%) (21). On the average, 2.67% and 2.85% of sinapine was found in the defatted rapeseed and canola cotyledons, respectively (22). Ismail and Eskin (23) employed a colorimetric method with titanium tetrachloride for quantitation of sinapine in rapeseed. The content of sinapine in rapeseed flour tested was 1.04% and rapeseed protein concentrates examined contained 0.11–0.18% sinapine.

Insoluble-bound phenolic acids. Rapeseed flours contained from 3.2–5.0 mg of insoluble-bound phenolic acids per 100 g of Yellow Sarson and Gorczanski rapeseed varieties, respectively (12). Krygier *et al.* (13) found no detectable amount of bound phenolics in Yellow Sarson, Candle and Tower flours, while Tower hulls contained 24.5 mg phenolics per 100 g sample. However, canola meals contained up to 100 mg of insoluble phenolic acids per 100 g sample (Table 2) (15). In total, nine phenolic acids were identified in the insoluble-bound fraction of phenolic acids; sinapic acid being the predominant phenolic acid in rapeseed flours, followed by *p*-coumaric and *trans*-ferulic acids (12). However, protocatechuic acid was reported to be a predominant phenolic acid in Tower hulls (13).

Sinapic acid constituted from 30.3 to 59.1% of the total insoluble-bound fraction of phenolic acids in rapeseed and mustard flours (12). On the other hand, the contribution of sinapic acid in cruciferae meals, based on the data from hexane extraction presented in Tables 2 and 3, ranged from 7.4% for Midas rapeseed to 32.1% for Domo mustard (18). The contribution of sinapic acid to the insoluble fraction of phenolic acids of Tower hulls was only 9.8% (13).

TANNINS (POLYPHENOLIC COMPOUNDS)

Condensed tannins in rapeseed hulls were first identified by Bate-Smith and Ribereau-Gayon (24). Later, Durkee (25) found pelargonidin, cyanidin and its *n*-butyl derivative in the hydrolytic products of rapeseed hulls. However, according to Leung *et al.* (26), leucocyanidin was the basic unit of tannins isolated from rapeseed hulls.

Clandinin and Heard (27) reported that rapeseed meal contained approximately 3% tannins as assayed by the AOAC (28) method of tannin determination in cloves and allspice. It has been shown, however, that this value included sinapine (29). Later, Fenwick *et al.* (30) reported whole and dehulled Tower meals to contain 2.71% and 3.91% tannins, respectively. On the other hand, the content of tannins assayable by the modified vanillin method (31) ranged from 0.09 to 0.39% in the defatted rapeseed cotyledons and from 0.23 to 0.54% in the defatted canola cotyledons (22). The meals obtained from canola varieties contained from 0.68 to 0.77% of condensed tannins. However, only 0.56% and 0.43% of tannins were present in high glucosinolate rapeseed variety Midas and Chinese cultivar Hu You 9, respectively (32–34). On the other hand, rapeseed hulls contained from 0.02 to 0.22% of extractable tannins (26,35). The apparent discrepancies in the reported data on tannin contents may be due to the existing differences in the solvent extraction systems employed for their recovery and methods subsequently used for their quantitation.

Gupta and Haslam (36) used water, methanol, ethanol, propanol, acetone and dimethylformamide for the extraction of sorghum polyphenolics, and found methanol to be the most efficient solvent system. However, Maxon and Rooney (37) and, later, Price *et al.* (30), suggested extracting sorghum tannins with methanol containing 1% concentrated HCl. In other work, 70% acetone was used for the extraction of tannins from rapeseed hulls (26). Naczk and Shahidi (38) found that the recovery yield of rapeseed tannins was affected by the presence of water, number of extraction steps and solvent-to-meal ratio. Pure solvents were poor extraction media for the isolation of tannins. Addition of water, up to 30% (vol/vol), improved the recovery of rapeseed tannins. They also found that a two-stage extraction was sufficient to recover soluble rapeseed tannins and that changing of the seed-to-solvent ratio from 1:5 to 1:10 increased the extraction of tannins by 70%

TABLE 4

Recovery of Rapeseed Tannins and Phenolics by Different Solvent Systems[a]

Solvent system	Total phenolics[b]	Tannins[c]
MeOH	402.3	35.1
70% MeOH	874.0	241.8
1% conc. HCl in 70% MeOH	1079.8	73.0
Acetone	66.0	0.0
70% Acetone	805.8	321.3
1% conc. HCl in 70% acetone	1010.7	216.9
DMF	554.2	0.0
70% DMF	1057.6	314.7
MeOH/acetone/H_2O (7:7:6)	999.8	221.9
Acetone/DMF/H_2O (7:7:6)	1112.0	312.9

[a]Reference 39.

[b]Expressed in mg sinapic acid per 100 g meal, on a dry basis.

[c]Expressed in mg catechin equivalents per 100 g meal, on a dry basis.

TABLE 5

Removal of Phenolic Acids and Tannins by Methanol-Ammonia-Water/Hexane Extraction[a]

Meal		Removal (%)	
		Phenolic acids	Tannins
Tower	A[b]	69.5	70.1
	B[c]	75.2	78.0
Regent	A	78.1	71.6
	B	74.6	77.5
Altex	A	71.1	72.6
	B	72.0	84.9

[a]From references 15 and 71. Reagent is *B. napus* canola.

[b]A, meal extracted with 10% NH_3 in methanol and hexane.

[c]B, meal extracted with 10% NH_3 in 95% methanol and hexane.

TABLE 6

The Content of Free, Esterified, Insoluble-Bound and Total Phenolic Acids in MeOH-NH_3-H_2O/Hexane Extracted *Brassica* Oilseeds[a]

Meal	Phenolic acids[b]			
	Free	Esterified	Insoluble-bound	Total
Midas	97.3	307.7	96.9	501.9
Triton	33.1	229.8	41.3	304.2
Mustard	32.2	221.4	45.1	298.6
Tower	105.0	202.0	76.0	383.0
Regent	135.0	228.0	103.0	466.0
Altex	137.0	253.0	115.0	505.0

[a]From references 15 and 18.

[b]Expressed as mg sinapic acid equivalents per 100 g meal, on a dry basis.

acetone, from 257.3 to 321.3 mg per 100 g of meal. Further changes in the ratio enhanced only the extraction of tannins by 70% methanol. On the other hand, addition of HCl reduced the recovery of tannins (39) (Table 4). The latter data are contrary to those reported by Price *et al.* (31) who found that addition of concentrated HCl to methanol improved the extraction of sorghum tannins. Thus, perhaps the nature of rapeseed tannins differs from those found in sorghum and beans (36,40).

EFFECT OF PROCESSING

Phenolic acids. Processing of rapeseed to protein concentrates and isolates, by typical procedures, reduced the content of phenolics by 60–83% (41). However, seven extractions with 70% ethanol at a solvent-to-meal ratio of 10:1 were required to obtain concentrates containing only trace levels of phenolics (12).

The content of phenolic compounds in oilseed meals may be reduced by treatment with ammonia (gaseous or in alkanol solution). McGregor *et al.* (42) found gaseous ammoniation of *Brassica juncea* mustard meal to remove up to 74% of sinapine. The treatment of *Brassica napus* meals with ammonia or lime reduced their sinapine content by about 90% (16,43). Extraction of Candle and Tower canola meals by ethanol containing 0.2M ammonia (44) removed up to 82% and 39% of their phenolics content, respectively. Authors of the latter article did not offer any explanation for the observed differences in the removal efficiency of phenolics from these meals.

The methanol-ammonia-water/hexane extraction system removed an average of 73.4% of the phenolics content of canola meals on a laboratory-scale (14) (Table 5), and up to 80% in a semi-pilot-scale extraction process (45). Data presented in Tables 2 and 6 indicate that on a percentage basis, the esterified phenolic acids are extracted more effectively than are the free phenolic acids. The removal of free phenolic acids ranged from 40.9% for Midas to 75.2% for Hu You 9; and the level of esterified phenolics ranged from 82.4% for Midas to 93.1% for Hu You 9 varieties. On the other hand, 34.6–73.6% and 83.8–91.4%, respectively, of the original sinapic acid in the free and in the esterified phenolic acid fractions of treated meals was removed. However, the content of the insoluble phenolic acid fraction and the contribution of sinapic acid to it was not affected to any great extent. The apparent increase in the content of insoluble-bound phenolic acids in methanol-ammonia-treated meals may be a direct result of the dissolution of nonprotein components of seed in the methanol-ammonia phase (15,32).

Tannins. Shahidi and Naczk (33) reported that methanol alone extracted only 16% of tannins present in rapeseed. Addition of 5% (vol/vol) water to methanol increased the efficiency of tannins extraction to 36%. Presence of ammonia in absolute or 95% methanol greatly enhanced the extraction of condensed tannins from rapeseed. Methanol-ammonia-water/hexane, however, was the most effective solvent system for the removal of tannins as compared with the methanol-ammonia/hexane system (Table 5). The resultant meals contained from 4 to 33% of condensed tannins originally present in rapeseed meals. This may be because of the extraction of tannins out of the seed and into the polar phase and/or their possible decomposition to products that are insensitive to vanillin reagent. Ghandi *et al.* (46) found ammonia to depolymerize the tannins present in salseed meal, thus producing a nontoxic and palatable meal. Moreover, upon alkali treatment, tannins may form phlobaphenes, which are chemically and nutritionally unreactive compounds (47). On the other hand,

TABLE 7

Effect of Phenolic Acid Combinations on Flavor Thresholds[a]

Compounds	Individual thresholds (ppm)	Combination thresholds (ppm)
Salicylic + *p*-hydroxybenzoic	(90); (40)	35
Salicylic + *p*-hydroxybenzoic + gentisic	(90); (40); (90)	40
Vanillic + *p*-hydroxybenzoic	(30); (40)	10
Vanillic + syringic	(30); (240)	90
Ferulic + *p*-coumaric	(90); (40)	25
Ferulic + gentisic	(90); (90)	80
Ferulic + gentisic + caffeic	(90); (90); (90)	60
Ferulic + gentisic + caffeic + syringic	(90); (90); (90); (240)	95

[a]Reference 49.

Fenwick *et al.* (43) reported no appreciable effect with ammonia or lime treatment on the content of tannins in *Brassica napus* meals.

EFFECTS OF PHENOLICS ON SENSORY PROPERTIES

Phenolic constituents may contribute objectionable flavors to some oilseeds (48), including sour, bitter, astringent and/or phenolic-like flavor characteristics. Taste thresholds for some individual phenolic acids present in oilseeds, including rapeseed, ranged from 30 ppm (protocatechuic acid) to 240 ppm (syringic acid). In this work, the taste threshold for sinapic acid was not determined due to its insolubility in water at the concentrations required for testing. A combination of phenolic acids resulted in much more sensitive thresholds than those for the individual acids (Table 7) (49,50). Results of this study revealed the contribution of free phenolic acids to the taste of rapeseed meals.

Sinapine is another bitter phenolic derivative component that is present in rapeseed meals at high concentrations. Therefore, it would also contribute to the reported unpleasant and bitter flavor of glucosinolate-free rapeseed flour (51). Moreover, it may have adverse effects on the palatability of rapeseed products (52). Sinapine also is linked to a crabby or fishy taint noted in eggs from some brown-egg laying hens (30,53,54), and serves as a precursor of trimethylamine (TMA) (55). The egg taint is caused by concentrations as low as 1 μg/g of TMA (54).

Some phenolic substances present in plants cause a puckering and drying sensation over the whole surface of the tongue and the buccal mucosa (56). This sensation is called astringency and is related to the ability of the substance to precipitate salivary proteins (57). According to Haslam (58), only tannins with a molecular weight ranging from 500 to 3,000 daltons may bring about the astringency sensation. Thus, an astringent phenol is a substance of moderate molecular size with a number of phenolic groups oriented into 1,2-dihydroxy or 1,2,3-trihydroxy configurations. At least two such orientations of phenolic groups are required in the molecule to impart astringency (59). Such phenolic substances bind to proteins more strongly than phenols with isolated hydroxyl groups (60). The phenol-protein complex can only precipitate when the complex becomes sufficiently hydrophobic. Molecular interpretations of chemical reactions responsible for astringency recently have been reported (61,62).

Declour *et al.* (63) determined the taste thresholds of astringency for tannic acid, (+)-catechin, procyanidin B-3 and mixtures of trimeric and tetrameric proanthocyanidins dissolved in deionized water. They found that these threshold values ranged from 4.1 to 46.1 mg/mL and that they were inversely proportional to the molecular weight of the phenolics.

EFFECTS OF PHENOLICS ON NUTRITIONAL PROPERTIES

General considerations. Phenolic acids can form complexes with proteins, thus lowering their nutritional value. Tannin-protein complexes may be responsible for the antinutritional effect of tannin-containing feeds that have been observed in both nonruminants (64,65) and ruminants (66). Mitaru *et al.* (35) reported that condensed tannins isolated from rapeseed hulls were not capable of inhibiting the activity of α-amylase enzyme *in vitro*. On the other hand, it has been postulated that tainting of eggs was due to the formation of rapeseed tannin-TMA oxidase complex (30,53,67). This enzyme converts TMA to odorless, water-soluble TMA oxide. However, the addition of extracted rapeseed tannins to soybean-containing diets for chicks resulted in reduction of their metabolizable energy, but did not have any apparent effect on the absorbability of proteins by chicks (68).

Formation of phenolic acid-protein complexes. Phenolic acids can form complexes with proteins, thus lowering their nutritional value. Loomis and Battaile (69) suggested that phenols can complex with proteins reversibly by a hydrogen-bonding mechanism or irreversibly by oxidation to quinones, which combine with reactive groups of protein molecules. Wade *et al.* (70) found binding of bovine serum albumin (BSA) to correlate well with the pK_a of simple phenols. Thus, the hydrogen bond between phenol and protein was stronger for more acidic phenols. Products of enzymatic and nonenzymatic oxidation of phenolics in seeds, meals or flours may readily react with the ε-NH_2 group of lysine and CH_3S group of methionine of enzymes and other proteins to form complexes, thus rendering them nutritionally unavailable to monogastric animals (71).

The possibility of phenolic-protein complex formation can be indirectly concluded from the amount of soluble matters extracted by 80% ethanol. Kozlowska and Zadernowski (72) reported quantities of extracted matters increased as the pH of 80% ethanol increased. The formation of these complexes also was investigated in model

systems consisting of sinapic acid and BSA protein by a fluorescence technique. The formation of complexes was favored in a neutral or alkaline pH (73).

Formation of tannin-protein complexes. Tannins may form soluble or insoluble complexes with proteins (74–76). The specificity of tannin-protein interaction depends on the size, conformation and charge of protein molecule (77). Proteins with a compact globular structure like ribonuclease, lysozyme or cytochrome C exhibit low affinity for tannins, whereas conformationally open proteins, such as gelatin and polyproline, readily form complexes with tannins. The precipitation of tannin-protein complex occurs because of the formation of a sufficiently hydrophobic surface on the complex (60). At low concentration of proteins the precipitation is due to formation of a hydrophobic monolayer of polyphenols on the protein surface. At higher concentrations of proteins, however, the hydrophobic surface results from combination of both complexing of polyphenol on the protein surface and cross-linking of different protein molecules with polyphenols. Thus, the stoichiometry of the protein-phenol complex depends on the protein concentration in solution. It is also possible to reverse the reaction of formation of the insoluble protein-phenol complex formation by the addition of an excess amount of protein (78). The lowest solubility of tannin-protein complex occurs at a pH near the isoelectric point of the protein (77,79). The tannin-protein interaction depends on the initial concentrations of both tannins and proteins. All proteins are precipitated when tannins are present in excess. When proteins are in excess, however, soluble protein-tannin complexes may be formed (60, 74,77).

The binding mechanism of proteins to tannins may be caused by the formation of multiple hydrogen bonds between the phenolic hydroxyl groups of tannins and the carbonyl functionalities of the peptide bonds of proteins (69,80,81). The tannin-protein complex also may be stabilized by other types of molecular interactions, such as ionic bonds between the phenolate anion and the cationic site of protein molecule (82), and/or covalent links formed as a result of condensation of oxidized phenolic groups of tannins with a nucleophilic group, such as SH, OH and NH_2 in the protein molecule (81,82,83), and/or hydrophobic interaction between the aromatic ring structure of tannin and hydrophobic region of proteins (82,84–86). The 1,2-di- (or 1,2,3-tri-) hydroxyphenyl residue is considered as the prime binding site of tannin. It is, however, believed that tannin-protein complexation is usually the result of formation of hydrogen bonds and hydrophobic interactions (77,85), particularly at acidic pH (87). Hagerman and Butler (88) reported that precipitation of tannin-protein was pH-sensitive; however, they did not observe any precipitation of proteins at pH values above the pK_a of phenolic groups. Based on these observations, they suggested that ionic bonds between protein and tannin moieties are less important.

The formation of a tannin-protein complex is not only affected by the composition and structure of proteins, but also by the size, length and flexibility of the tannin molecule. It has been demonstrated that tannins should have at least three flavanol subunits to be effective protein-precipitating agents. Dimers did precipitate proteins, but were much less effective, and simple flavanols did not precipitate proteins at all (57,89,90). Similarly, Porter and Woodruffe (91) reported the formation of insoluble complexes between proanthocyanidins and proteins (hemoglobin) to depend more on the molecular weight of tannins than on the configuration and the number of hydroxyl groups on the B-ring. However, the configuration and the number of hydroxyl groups on the B-ring may also affect the ability of tannins to precipitate proteins. It was demonstrated that flavanols with three ortho-hydroxy groups, like prodelphidins, bind proteins more tightly than do those with two ortho-hydroxy groups on the B-ring, as in procyanidins (92,93).

The reduction of food intake and growth of experimental animals (rats and chicks), as well as a decrease in protein digestibility (94,95) have been associated with the ingestion of polyphenols (96,07). Mitjavila *et al.* (98) reported increased fecal calcium losses by rats fed with 3% tannic acid as result of increased endogenous gut secretions. Marquardt and Ward (99) observed that tannins from fababeans accounted for about 50% growth depression of chicks. The tannin content was highly correlated with a decrease in weight gain, decreased feed intake, decreased retention of protein and increased fat retention. Also, the utilization of barley proteins by rats was negatively correlated with the presence of 0.55–1.23% of tannins in barley samples (100). The reduction in utilization of proteins may be due to binding of tannins to digestive enzymes and/or to dietary proteins. Condensed tannins may also bind to methionine, thus making it unavailable, hence lowering utilization of dietary proteins (101,102).

In addition to sinapine, tannins in rapeseed meal are also implicated in tainting of eggs. It is postulated that they block metabolism of TMA by inhibiting TMA oxidase (30,53,67). This enzyme converts TMA to odorless, water-soluble TMA oxide. The addition of tannins, extracted from rapeseed meal, to soybean-containing diet for chicks, resulted in reduction of its metabolizable energy, but did not have any apparent effect on the absorbability of proteins by chicks (68). However, Mitaru *et al.* (35) reported that condensed tannins isolated from rapeseed hulls were not capable of inhibiting the activity of α-amylase enzyme *in vitro*. Some studies under *in vitro* conditions indicated that tannins may even show a stimulatory, rather than an inhibitory, effect on protein digestion (55,75). This may be due to partial denaturation of the protein substrate.

Interaction with carbohydrates. Polyphenols also may form complexes with carbohydrates. The affinity for polysaccharides is strongly dependent on the molecular size, conformational mobility and shape, as well as water solubility of polyphenols. Thus, an increase in molecular size and conformational flexibility of tannins enhances the affinity of tannins for carbohydrates (103).

Davis and Harbers (104) found starch obtained by wet milling (from bird-resistant sorghum) was less susceptible to enzyme attack than other similarly isolated sorghum starches. They suggested that this was due to adsorption and retention of condensed tannins on starch. Later, Davis and Hoseney (105) reported 40–60% of tannins were bound by starch and this depended on the source of tannins as well as the starch species. They also noticed at least two fractions of condensed tannins in an α-amylase-inhibiting fraction of grain sorghum that was adsorbed on starch and an inhibiting fraction that was not adsorbed on starch. Also, Desphande and Salunkhe (106)

TABLE 8

Binding of Tannic Acid to Different Starches and Starch Fractions (μg catechin equivalents per 100 mg of starch)[a]

Conditions	Starch/starch fraction				
	Split yellow pea	Small red bean	Potato starch	Amylose	Amylopectin
21°C for 4 h	522	261	358	652	587
95°C for 0.5 h	394	278	267	186	214

[a]Reference 106.

studied the interaction of tannic acid and catechin with five different legume starches, as well as with potato starch in model systems. Up to 652 μg of tannic acid and up to 586 μg of catechin were interacting with each 100 mg of starch. They also found heating for 0.5 h at 95°C substantially reduced the ability of starches to complex with either tannic acid or catechin (Table 8). Some phenolic acids may have flatulence-inhibiting properties. Rackis *et al.* (107) reported phenolic acids such as syringic and ferulic acids inhibited flatulence from soybean meals both in *in vitro* and *in vivo* studies.

Interaction with minerals and other food constituents. Tannins may precipiate a wide range of essential minerals, thus lowering their bioavailability (19). They may form insoluble complexes with divalent metallic ions, thus lowering their absorption.

Phenolic compounds have been identified as possible inhibitors of iron absorption (108,109). This inhibition may be caused by formation of insoluble iron-phenol complexes in the gastrointestinal tract, thus making the iron unavailable for absorption. Brune *et al.* (110) suggested that phenolic compounds with galloyl groups are mainly responsible for inhibition of iron absorption. They also found a relationship between the content of galloyl groups in foods and the degree of inhibition of iron absorption. However, phenolic compounds with at least two adjacent hydroxy groups (bearing catechol groups or galloyl groups) may have marked iron-binding properties (111).

Caffeic acid and tea flavonoids have been reported to have antithiamine effects. Formation of thiamine-phenol complexes adversely affects the availability of thiamine. An oxidation process is probably involved because ascorbic acid prevented this complexation (112,113). Tannic acid was found to precipitate vitamin B_{12}, thus making it unavailable and contributing to anemia (114).

Future research needs. Phenolic compounds not only affect the taste of rapeseed protein products, but also lower their nutritional value. However, the available information on the undesirable properties of rapeseed/canola phenolic compounds is still fragmentary. Therefore, more detailed studies on the nutritional implications caused by interaction with phenolic compounds of rapeseed food components and their sensory effects are required.

The variability of the reported results on tannin content in rapeseed/canola is caused by the existing differences in the solvent-extraction systems employed for their recovery and quantitation methods used. Therefore, there is a need to examine and to develop more efficient solvent-extraction systems and to standardize the extraction conditions used for the recovery of tannins. Different methodologies for tannin determination are described in the literature (115,116); however, no systematic and in-depth studies regarding the suitability of these methods for determination of tannins in rapeseed/canola have been carried out.

The specificity of tannin-protein interactions has been well documented for a number of plant tannins (85,117). However, little is known about the tannin-protein interaction in rapeseed/canola. Variability in the affinity of rapeseed/canola tannins to different proteins requires further study.

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