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Current research developments on polyphenolics of rapeseed/canola: a review

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The utilization of rapeseed/canola as a source of food-grade proteins is still limited due the presence of glucosinolates, phytates, hulls and phenolics. Phenolic acids and condensed tannins are the predominant phenolic compounds found in rapeseed. The content of phenolic compounds in rapeseed/canola products is much higher than that found in corresponding products from other oleaginous seeds. Phenolics such as free phenolic acids, sinapines and condensed tannins may contribute to the bitter taste and astringency of rapeseed products. In addition, both phenolic acids and condensed tannins may form complexes with proteins, thus lowering the nutritional value of rapeseed products. The specific mode of interaction of rapeseed phenolics with proteins is still not well understood. Therefore, a better knowledge of factors which influence the interactions between phenolics and proteins would be beneficial in developing more efficient technologies for production of phenolic-free rapeseed protein isolates. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The global production of rapeseed, including canola varieties, ranks third amongst oilseed crops (Shahidi, 1990). Rapeseed is conventionally processed to oil and meal by employing an extraction process which is an adaptation of soybean technology adjusted to high oil content, small seed size and presence of glucosinolates (Unger, 1990). The meal obtained after oil extraction contains about 40% protein. The quality of rapeseed protein as represented by its amino acid composition is well-balanced for human use (Ohlson, 1978). Shahidi and Naczk (1992) reported that amino acids of canola were not sensitive to commercial processing or extraction with methanol-ammonia/hexane. Delisle *et al.* (1984) reported that the protein efficiency ratios of rapeseed and soybean meals were 2.64 and 2.19, respectively. However, the use of rapeseed as a source of food protein is still thwarted by the presence of undesirable components such as glucosinolates, phytates, hulls, and phenolics.

The composition of rapeseed has been significantly altered by the Canadian breeders who have developed new varieties of rapeseed, known as canola. The content of glucosinolates in canola has been significantly reduced (almost ten-fold) but, although the new varieties may be used in animal feed, they still contain levels of glucosinolates too high to be considered as a suitable protein source in human food products. Glucosinolates in the meal may undergo decomposition to toxic compounds, such as isothiocyanates, nitriles, and thiocyanates, by microorganisms in the lower gastrointestinal tract (Oginsky *et al.*, 1965; Nishie and Daxenbichler, 1980; Fenwick *et al.*, 1986; Rabot *et al.*, 1995). Therefore, a number of new processes have been developed to reduce the content of glucosinolates in the meal. These methods involve chemical, microbial and physical treatments of meals or seeds (Fenwick *et al.*, 1986; Rubin *et al.*, 1986; Shahidi and Naczk, 1990). Despite these developments, the removal of glucosinolates is still not a standard practice in rapeseed/canola oil extraction plants.

Hexane-extracted canola/rapeseed meals contained between 11.3 and 14.3 mg phosphorus per g of meal. In these meals, phytic acid represents the major source of total phosphorus (78.6 to 88.1%) (Naczk *et al.*, 1986). Phytic acid functions as the storage form of phosphate and inositol in the mature seeds. In foods and feeds, phytic acid can bind mono and divalent metal ions to form complex phytates, thus reducing their bioavailability

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(Erdman, 1979; Cheryan, 1980; Cosgrove, 1980; Maga, 1982; Morris, 1986; Hallberg, 1987; Thompson, 1990). A reduction in the bioavailability of several metals, notably zinc, in the presence of phytic acid has been reported (Erdman, 1979; Jones, 1979; Cheryan, 1980; Maga, 1982).

The content of hulls in rapeseed varies from 10.5 to 20% of the seed weight and 20 to 30% of the defatted meal, on a dry weight basis (Applequist and Ohlson, 1972; Theander *et al.*, 1977; Bell, 1993; Jensen *et al.*, 1995). The hulls consist of low molecular weight carbohydrates, polysaccharides, pectins, cellulose, lignin, as well as proteins, polyphenolics, glucosinolates and minerals. The presence of high levels of indigestible hull limits the use of rapeseed meal in some feed formulations (Liesle *et al.*, 1973; Jones and Sibbald, 1979; Sarwar *et al.*, 1981) as occurrence of undigested hulls in animal extracts is interpreted by some farmers as an indication that rapeseed products are poorly digested. Therefore, a number of processes for dehulling of rapeseed/canola have been proposed. The removal of hulls can be accomplished by air-classification of the defatted meal (Tape *et al.*, 1970; Diosady *et al.*, 1986), by liquid cyclone fractionation after solvent extraction (Sosulski and Zadernowski, 1981) or by cracking and air-classification of seeds before oil extraction (Jones, 1979; Schneider, 1979; Jones and Holme, 1982; Baudet *et al.*, 1983). The mechanical methods for separation of hulls from canola/rapeseed are still inefficient and therefore dehulling is not a standard practice in canola oil extraction plants.

The predominant phenolics present in rapeseed/canola are phenolic acids and condensed tannins. The content of phenolic acids in rapeseed flour (dehulled, defatted rapeseed) is from 10 to 30% higher than that found in flours obtained from other oleaginous seeds. However, the content of phenolic acids in rapeseed meal (defatted whole rapeseed) is up to five times higher than those found in soybean meals (Table 1). Therefore phenolics are important factors when considering canola/rapeseed meal as a source of human food-grade protein because they contribute to the dark colour, bitter taste, and astringency of rapeseed protein products (Clandinin, 1961; Malcolmson *et al.*, 1978; Sosulski, 1979; Ismail *et al.*, 1981; Shahidi and Naczk, 1992). In addition, phenolics and their oxidized products can form complexes with essential amino acids and proteins, thus lowering the nutritional value of rapeseed products. This review discusses some technological implications of rapeseed phenolics.

Table 1. Total content of phenolic acids in some oilseed products

Oilseed product	($g\,kg^{-1}$ dry basis)
Soybean flour[a]	0.23
Cottonseed flour[a]	0.57
Peanut flour[a]	0.63
Rapeseed/canola flour[a]	6.4–12.8
Canola meal[b]	15.4–18.4
Soybean meal[b]	4.6

[a]Adapted from Kozlowska *et al.*, 1991.
[b]Adapted from Naczk *et al.*, 1986.

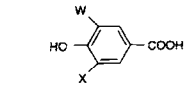
LOCALIZATION AND SOME FUNCTIONS OF PHENOLIC COMPOUNDS IN SEEDS

The presence of phenolic compounds in the nucleus of *Brassica napus* was first reported by Wronka *et al.* (1994). These authors suggested that phenolic compounds may play an important regulatory role in the seed nucleus. The content of phenolic compounds in rapeseed increases during the ripening of seeds. The content of water-soluble phenolics, including sinapine, is maximum between the final stage of the green seeds and the beginning of their browning, reaching a stable level at the stage of ripeness. In contrast, the content of diethyl ether-soluble phenolics may or may not increase during seed maturation, depending on the rapeseed variety examined (Rotkiewicz *et al.*, 1987). The localization of tissues containing phenolics changes during the maturation of embryos. In the ovary containing a globular embryo, the phenolics are located in the epidermis of the *integumentum externum* and in the innermost layer of the *integumentum internum*. In the green, hard seeds, the tissue remaining after the *integumentum internum* and the layers over the stellar cells have disappeared, contains cell walls impregnated with phenolics. Mature, black seeds contain only one distinct layer of stellar cells, which, like other compressed cell walls, are impregnated with phenolics (Iwanowska *et al.*, 1994). The embryos of dry *Brassica napus* seeds contain phenolic compounds on the surface of the root cap in the space between the plasmalemma and the cell walls. The imbibition of dry seeds promotes the extrusion of phenolics outside the plasma membrane (Wronka *et al.*, 1994). During the germination of seedlings, the sinapine, the most abundant phenolic acid ester found in rapeseed, is hydrolysed to choline and sinapine acid (Tzagoloff, 1963). Sinapine acid is then used for the biosynthesis of lignins and flavonoids (Neish, 1960) and choline is an important substrate in the methylation cycle (Byerrum and Wing, 1953).

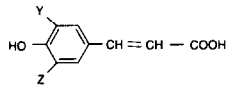
PHENOLIC ACIDS CONTENT

Total content

Phenolic acids in rapeseed are present in the free, esterified and insoluble-bound forms and are derivatives of benzoic and cinnamic acids. The chemical structures of main phenolic acids of canola/rapeseed are shown in Fig. 1. *Trans*-sinapic acid is the predominant phenolic acid found in rapeseed/canola. The presence of *cis*-sinapic acid in rapeseed/canola products is the result of isomerization that may be brought about by the



Acid	W	X
Protocatechuic	H	OH
Vanillic	OCH_3	H
Syringic	OCH_3	OCH_3
Gallic	OH	OH
p-Hydroxybenzoic	H	H



Acid	Y	Z
p-Coumaric	H	H
Caffeic	H	OH
Ferulic	H	OCH_3
Sinapic	OCH_3	OCH_3

Fig. 1. Structures of phenolic acids found in canola and rapeseed.

exposure of seeds and/or rapeseed products to UV radiation (Schulz and Hermann, 1980). The total content of phenolic acids in rapeseed meals is up to 18.4 g kg^{-1}, on a dry weight basis, of defatted meal. In contrast, rapeseed flours contain from approximately 6.2 to 12.8 g of total phenolic acids per kg, on a dry weight basis (Kozlowska *et al.*, 1975, 1983; Krygier *et al.*, 1982; Naczk and Shahidi, 1989; Naczk *et al.*, 1992).

Free phenolic acids

Rapeseed meals may contain over 2 g of free phenolic acids per kg meal (Naczk and Shahidi, 1989; Naczk *et al.*, 1992), whereas the content of free phenolics in rapeseed flours may range from trace quantities to 1 g kg^{-1} (Krygier *et al.*, 1982; Kozlowska *et al.*, 1983) (Table 2). Sinapic acid constitutes from 70 to 85% of the total free phenolic acids (Table 3). Rapeseed/canola products also contain small quantities of *p*-hydroxybenzoic, vanillic, gentisic, protocatechuic, syringic, *p*-coumaric, *cis*- and *trans*-ferulic, caffeic and chlorogenic acids in the free form (Krygier *et al.*, 1982; Kozlowska *et al.*, 1991).

Esterified phenolic acids

Esterified phenolic acids are the predominant fraction of phenolic acids present in rapeseed protein products as they constitute up to 80% of the total phenolic acids. Rapeseed flours contain more phenolic acids in the form of esters than rapeseed meals (Table 2). Flours obtained from Tower and Candle varieties of canola contained 9.8 and 12.0 g phenolic acids liberated from esters per kg, respectively (Krygier *et al.*, 1982) while those obtained from Polish rapeseed varieties (Start, Gorczanski and Bronowski) contained 5.2–7.0 g phenolic acids per kg (Kozlowska *et al.*, 1983). However, meals obtained from Tower, Regent and Altex canola contained up to 14.6 g per kg of phenolic acids liberated from esters (Naczk and Shahidi, 1989). Sinapic acid constituted 71–97% of the phenolic acids liberated from the soluble fraction of esterified phenolic acids in rapeseed/canola meals (Table 3). The alkaline hydrolysate of the phenolic esters also contained small quantities of *p*-hydroxybenzoic, vanillic, protocatechuic, syringic, *p*-coumaric, *cis*- and *trans*-ferulic, and caffeic acids liberated from their esters (Krygier *et al.*, 1982).

Table 2. Content of free, esterified, and insoluble-bound phenolic acids in some rapeseed products

Product	Phenolic acids (g kg^{-1}, dry basis)		
	Free	Esterified	Insoluble-bound
Tower meal[d]	2.44	12.0	0.96
Regent meal[d]	2.62	14.7	1.05
Altex meal[d]	2.48	14.6	1.01
Tower flour[b]	0.98	9.8	—
Candle flour[b]	0.84	12.0	—
Gorczanski flour[c]	0.60	5.7	0.05
Start flour[c]	0.72	7.0	0.05
Hu You 9 meal[d]	1.19	11.8	0.39
Midas meal[d]	1.44	15.2	0.69
Triton meal[d]	0.62	12.1	0.51

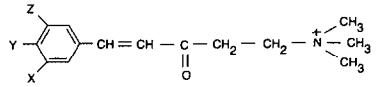
[a]Adapted from Krygier *et al.*, 1982.
[b]Adapted from Kozlowska *et al.*, 1983.
[c]Adapted from Kozlowska *et al.*, 1975.
[d]Adapted from Naczk and Shahidi, 1989.

Table 3. Contribution of sinapic acid to the content of free, esterified and insoluble-bound phenolic acids in some rapeseed products

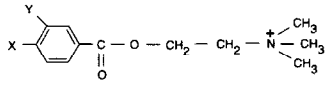
Meal	% of the total phenolic acids		
	Free	Esterified	Insoluble-bound
Hu You 9	76.9	96.4	17.0
Midas	72.1	70.9	7.2
Triton	70.2	96.7	15.5

Adapted from (Naczk *et al.*, 1992).

A number of phenolic choline esters, known as sinapines (Fig. 2), have been found in seeds of the *Brassicaceae* family. An excellent review of sinapines in rapeseed has recently appeared (Pokorny and Reblova, 1995). According to Bouchereau *et al.* (1991), the composition of phenolic acids in sinapines is genetically controlled, but their content is affected both by the cultivar and growing conditions. The presence of sinapines such as sinapoylcholine (sinapine), feruloylcholine, isoferuloylcholine, coumaroylcholine, 4-hydroxybenzoylcholine and 3,4-dimethoxybenzoylcholine was reported in rapeseed (Larsen *et al.*, 1983). Of these, sinapine, the choline ester of sinapic acid, was the most abundant phenolic choline ester present. *Brassica napus* cultivars contained higher amounts of sinapine (1.65–2.26%) than *Brassica campestris* cultivars (1.22–1.54%) (Mueller *et al.*, 1978). Later, Clausen *et al.* (1985) reported that the content of sinapine in *Brassica napus* cultivars ranged from 0.62 to 1.06% while that of *Brassica campestris* cultivars was 0.39–0.76% (Table 4). Defatted rapeseed and canola cotyledons contained, on average, 2.67 and 2.85% of sinapine, respectively (Blair and Reichert, 1984). In contrast, hulls of rape and canola seeds contained from 0.06 to 0.24% sinapine



Sinapines	X	Y	Z
Coumaroylcholine	H	OH	H
Feruloylcholine	H	OH	OCH_3
Isoferuloylcholine	H	OCH_3	OH
Sinapine	OCH_3	OH	OCH_3
Sinapine glucoside	OCH_3	O-Glu	OCH_3



Sinapines	X	Y
4-Hydroxybenzoylcholine	OH	H
Hesperalin	OCH_3	OCH_3

Fig. 2. Structures of sinapines found in canola and rapeseed.

Table 4. Sinapine content ranges in some rapeseed/canola cultivars

Cultivar	Content (%)
Candle	0.39–0.76
Tobin	0.57–0.69
Altex	0.62–0.77
Regent	0.74–0.84
Tower	0.72–0.79
Line	0.79–1.06
Karat	0.81–0.98

Adapted from Pokorny and Reblova (1995).

(Bell and Shires, 1982). The diversity of the available data on sinapine contents may be due to the biological factors (stage of ripeness, growing locations, cultivars), the differences in solvent systems employed for their recovery and the methods used for their quantitation.

Sinapines are not the only phenolic esters found in rapeseed. Fenton *et al.* (1980) isolated sinapine and at least seven other phenolic esters from rapeseed meals of Midas and Echo varieties that upon hydrolysis yielded sinapic acid. The presence of methyl esters of *cis*- and *trans*-ferulic acids in rapeseed was confirmed by mass spectrometry (Amarowicz *et al.*, unpublished results). In addition, Zadernowski (1987) found that flours obtained from Polish rapeseed varieties contained from 0.16 to 0.65 mmol phenolic acid glycosides per kg of flour. Later, Wanasundara *et al.* (1994), Amarowicz and Shahidi (1994) and Amarowicz *et al.* (1995*b*) reported that 1-O-β-D glucopyranosyl sinapate was the predominant glycoside found in rapeseed/canola varieties. Presence of flavonoid glycosides containing sinapic acid bound to aglycone was first observed by Durkee and Harborne (1973). Later, Tantawy *et al.* (1983) reported the presence of two flavonoid glycosides, namely 3-(O-sinapoyl sophoroside)-7-O-glucoside of kaempferol and 3-(O-sinapoyl glucoside) 7-O-sophoroside of kaempferol in rapeseed meal.

Insoluble-bound phenolic acids

Canola meals contain approximately 1 g insoluble-bound phenolic acids per kg of meal (Naczk and Shahidi, 1989). In contrast, rapeseed flours contain only from 32–50 mg of insoluble-bound phenolic acids per kg (Kozlowska *et al.*, 1983). However, Krygier *et al.* (1982) reported that Yellow Sarson, Candle and Tower flours did not contain any detectable quantity of insoluble-bound phenolics. Nine phenolic acids were identified in the fraction of insoluble-bound phenolic acids isolated from rapeseed flours. Sinapic acid was found to be a predominant phenolic acid as it constituted 30 to 59% of the total insoluble fraction of phenolic acids in rapeseed and mustard flours (Durkee and Thivierge, 1975; Kozlowska *et al.*, 1983). In contrast, in *Cruciferae* meals, the contribution of sinapic acid to the insoluble-bound phenolic acids fraction ranged from 7.4%, for Midas variety, to 32.1%, for mustard seed of Domo variety (Table 3) (Naczk *et al.*, 1992). Tower canola hulls contained 245 mg insoluble-bound phenolics per kg of hulls. Protocatechuic acid was found to be the predominant insoluble-bound phenolic acid in Tower canola hulls. The contribution of sinapic acid to the insoluble fraction of hull's phenolic acids was only 9.8% (Krygier *et al.*, 1982).

CONDENSED TANNINS CONTENT

Tannins are complex phenolic compounds having a molecular weight in the range 500–3000 Da. They are classified either as condensed or hydrolysable, based on their structural types and their reactivity towards hydrolytic agents, particularly acids. Hydrolysable tannins are phenolics that, upon acidic, alkaline or enzymatic hydrolysis, produce a polyhydroxy moiety (usually D-glucose) and phenolic acids such as gallic acid and/or hexahydroxydiphenic acid. The latter acid, upon lactonization, produces ellagic acid. However, condensed tannins are dimers, oligomers and polymers of flavan-3-ols which, upon acidic hydrolysis (usually by using butanol-HCl), produce anthocyanidins and are therefore also known as proanthocyanidins.

Condensed tannins were first identified in rapeseed hulls by Bate-Smith and Ribereau-Gayon (1959). Later, Durkee (1971) verified this finding and identified cyanidin, pelargonidin and an artefact, the n-butyl derivative of cyanidin, in the hydrolytic products of rapeseed hulls. However, according to Leung *et al.* (1979) leucocyanidin is a basic unit of tannins isolated from rapeseed hulls (Fig. 3).

Several colorimetric methods have been developed for quantitation of tannins, but only a few are specific for condensed tannins. These include the modified vanillin assay of Price *et al.* (1978), the proanthocyanidin assay (Porter *et al.*, 1986; Mole and Waterman, 1987) and the 4-(dimethylamino)cinnamaldehyde (DAC) assay (McMurrough and McDowell, 1978; Thies and Fischer,

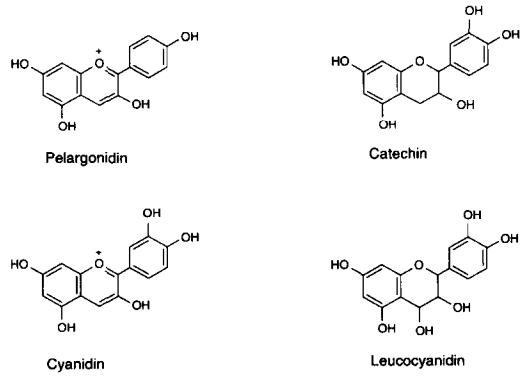


Fig. 3. Structures of basic units of condensed tannins of rapeseed.

1979). Of these, the vanillin method is widely used for quantitation of condensed tannins due to its specificity for flavanols and dihydrochalcones, both of which possess 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 and Howarth, 1976). Recently, Naczk *et al.* (1994) reported the existence of statistically significant ($P < 0.001$) linear relationships (correlation coefficients, $r^2 > 0.96$) between the tannin contents determined by the vanillin and the proanthocyanidin and DAC (4-(dimethylamino)cinnamaldehyde) assays (Fig. 4).

Rapeseed meal contains approximately 3% tannins as assayed by the AOAC method for the determination of tannins of cloves and allspice (AOAC, 1965; Clandinin and Heard, 1968). However, it has been shown that this value included sinapine (Fenwick and Hoggan, 1976). Later, Fenwick *et al.* (1984) reported that whole and dehulled Tower meals contain 2.71 and 3.91% tannins, respectively. The content of tannins assayed by the

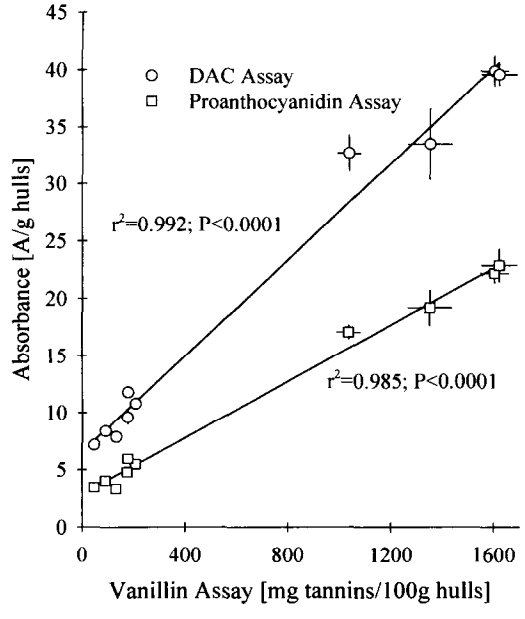


Fig. 4. Linear relationship between different chemical assays used for quantification of canola tannins (adapted from McDonald *et al.*, 1992).

modified vanillin method only ranged from 0.09–0.39% in the defatted rapeseed cotyledons and from 0.23–0.54% in the defatted canola cotyledons (Blair and Reichert, 1984). Shahidi and Naczk (1988, 1989) found that canola meals contained 0.68–0.77% of condensed tannins. The discrepancies in the reported data on tannin contents may be due to the different solvent systems employed for the extraction and the methods used for the quantitation of tannins.

Mitaru *et al.* (1982) reported that rapeseed hulls contain 0.02–0.22% condensed tannins. According to Leung *et al.* (1979), rapeseed hulls contain no more than 0.1% condensed tannin extractable by solvent systems commonly used for isolation of polyphenols. Recently, Naczk *et al.* (1994) and Naczk and Shahidi (1995) reported that the content of tannins in rapeseed/canola hulls ranged from 0.14 to 23 g tannin per kg hulls (Table 5). They also found that tannin contents within a particular canola cultivar varied by a factor of 9 to 15. These data indicate that both cultivar differences and environmental growing conditions may influence the content of condensed tannins in canola hulls. In addition, they reported that crude tannin extracts from canola hulls contained approximately 20% proanthocyanidins which were soluble in ethyl acetate. According to Porter (1989), only monomeric and dimeric proanthocyanidins are highly soluble in ethyl acetate. More

Table 5. Content of condensed tannins in rapeseed/canola hulls as determined by the vanillin and proanthocyanidin assays (g tannins[a] per kg hulls)

Hull sample	Vanillin assay	Proanthocyanidin assay
Rapeseed varieties[b]		
Polo	15.1	8.4
Mar	1.3	2.1
Marita	1.2	2.0
Honk	0.6	—
Idol	0.9	—
Spring turnip Rapeseed varieties[b]		
Dalstr 1	1.9	—
Dalstr 3	5.9	—
Canola varieties		
Cyclone sample 1[b]	17.2	11.0
Sample 2[b]	5.9	3.3
Sample 3[b]	11.2	7.6
Sample 4[d]	7.8	—
Sample 5[d]	20.8	—
Sample 6[c]	14.0	9.6
Westar sample 1[c]	2.1	2.9
Sample 2[c]	1.1	1.3
Sample 3[c]	16.5	11.1
Sample 4	0.9	1.9
Delta[c]	0.5	1.6
Excel[c]	1.7	3.0

[a]Purified tannins isolated from Cyclone hulls, sample 1, were used as standard.
[b]From Naczk, unpublished results.
[c]Adapted from Naczk *et al.*, 1994.
[d]Adapted from Naczk *et al.*, 1996.

detailed research is still needed to determine the effect of growing conditions on tannin contents of rapeseed/canola hulls.

ORGANOLEPTIC EFFECTS

Malcolmson *et al.* (1978) described the taste of water slurries of rapeseed protein concentrates as being bitter, astringent and unpleasant. A number of authors have suggested that phenolic compounds may contribute to these objectionable flavours. Arai *et al.* (1966) described the taste characteristics of oilseed phenolic acids as sour, astringent, bitter and/or phenol-like. Maga and Lorenz (1973) determined the taste thresholds for some individual phenolic acids present in oilseeds including rapeseed. They found that these thresholds ranged from 30 mg l^{-1} (vanillic acid) to 240 mg l^{-1} (syringic acid). However, the taste threshold for sinapine acid was not determined due to its insolubility in water at the concentrations required for testing. Combinations of phenolic acids resulted in much lower thresholds than those of their individual components (Table 6). Rapeseed meals may contain over 2 g of free phenolic acids per kg meal (Naczk and Shahidi, 1989; Naczk *et al.*, 1992), while for rapeseed flours the reported values range from trace quantities to 1 g per kg (Table 2) (Kozlowska *et al.*, 1983; Krygier *et al.*, 1982). The results of these studies indicate that free phenolic acids should contribute to the taste of rapeseed meals.

Sinapine, the most abundant phenolic ester in rapeseed, is a bitter tasting phenolic compound which is widely distributed among members of the *Cruciferae* family and therefore it would also contribute to the unpleasant and bitter flavour of glucosinolate-free rapeseed products (Clandinin, 1961; Sosulski, 1979). Ismail *et al.* (1981) have demonstrated that, not only sinapine, but also its components may contribute to the unpleasant taste of rapeseed protein products. They found, using the magnitude estimation test, that choline chloride and sinapic acid accounted for 80% of the bitterness of sinapine at equimolar concentrations. In addition, they demonstrated that 50 to 94% of the bitterness perceived from tasting water slurries of rapeseed flours and concentrates could be explained by the bitterness expected from their content of sinapine and choline chloride. However, sinapine is not the only bitter tasting choline ester found in rapeseed. According to Larsen *et al.* (1983), other phenolic choline esters may also contribute to the unpleasant taste of rapeseed products (Fig. 2). Sinapine is also linked to a crabby or fishy taint noted in eggs from some brown-egg laying hens (Hobson-Frohock *et al.*, 1973; Butler *et al.*, 1982; Fenwick *et al.*, 1979, 1984) as a precursor of trimethylamine (TMA) (Pearson *et al.*, 1980). The egg-taint is caused by low concentrations (about 1 g kg^{-1}) of TMA (Hobson-Frohock *et al.*, 1973). TMA accumulates, probably as a result of inhibition of trimethylamine oxidase activity by sinapine (Goh *et al.*, 1979) and/or degradation products of glucosinolates (Fenwick *et al.*, 1980; Goh *et al.*, 1985).

Table 6. Effect of phenolic acid combinations on flavour thresholds (mg l^{-1})[a]

Phenolic acid	Individual thresholds	Combination thresholds
Salicylic + *p*-hydroxybenzoic	(90);(40)	35
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

[a]Phenolic acids are used in equal amounts. Adapted from Maga and Lorenz (1973).

Some phenolic substances present in plants are able to bring about a puckering and drying sensation over the whole surface of the tongue and the buccal mucosa, called astringency (Lea and Arnold, 1978; Haslam and Lilley, 1988). This sensation is related to the ability of substance to precipitate salivary proteins (Bate-Smith, 1973). According to Haslam (1981), only tannins with molecular weights ranging from 500 to 3000 Da may bring about the astringency sensation. Delcour *et al.* (1984) determined the thresholds of astringency of tannic acid, (+)-catechin, procyanidin B-3, and mixtures of trimeric and tetrameric proanthocyanidins dissolved in deionized water. These threshold values range from 4.1 to 46.1 mg l^{-1}. They also found that the higher molecular weight substances had a lower threshold value. Rapeseed hulls contained up to 23 g condensed tannins kg^{-1} (Naczk *et al.*, 1994; Naczk and Shahidi, 1995). Thus, the condensed tannins present in hulls may contribute to the astringent taste of rapeseed meals.

Phenolic compounds and choline are not the only rapeseed constituents suggested as precursors of unpleasant taste in rapeseed products. Donaldson *et al.* (1978) reported that rapeseed protein concentrates contained sub-threshold levels of bitter tasting amino acids in the free form. Moreover, residual amounts of glucosinolate degradation products may contribute to the bitterness of rapeseed products, as at least one of these, goitrin, has been found to be bitter (Boyd, 1950). Thus, the bitterness of rapeseed products may result from both additivity and some synergism among the bitter stimuli present at both sub-threshold and supra-threshold levels. Such interactions have been demonstrated using model systems for both binary and quaternary mixtures (Bartoshuk and Cleveland, 1977).

NUTRITIONAL EFFECTS

Interaction with minerals

Phenolic compounds have been identified as possible inhibitors of iron absorption (Hallberg and Rossander,

1982; Gillooly *et al.*, 1983). This inhibition may be due to the formation of insoluble iron-phenol complexes in the gastrointestinal tract, thus making the iron unavailable for absorption. Brune *et al.* (1989,1991) suggested that phenolic compounds with a galloyl groups are mainly responsible for inhibition of iron absorption. They found a relationship between the content of galloyl groups in foods and the degree of inhibition of iron absorption. Recently, Naczk and Shahidi (1995) have determined the content of condensed tannins and iron-binding phenolics in selected canola varieties developed within the Canadian breeding program. The content of condensed tannins ranged from 3.6 to 6.9 g catechin equivalents per kg meal, while the content of iron-binding phenolics ranged from 1.6 to 4.5 g catechin equivalents per kg meal. They found that a statistically significant ($p < 0.005$) linear relationship ($r^2 = 0.76$) existed between the content of condensed tannins and iron-binding phenolics (Fig. 5). Therefore, removal of condensed tannins may improve the nutritional value of canola/rapeseed protein products.

Interactions with proteins: general considerations

Phenolics can form complexes with proteins, thus lowering their nutritional values. Loomis and Battaile (1966) suggested that phenols can complex with proteins reversibly by hydrogen bonding between the hydroxyl groups of phenols and the carbonyl functionalities of the peptide bonds of proteins or irreversibly by oxidation to quinones which combine with reactive groups of protein molecules. Wada *et al.* (1969) found that the binding of serum albumin correlated well with pK_a values of simple phenols. The phenol–protein complex may also be stabilized by ionic bonds between the phenolate anion and the cationic site of protein molecules (Wada *et al.*, 1969; Loomis, 1974) and/or hydrophobic interaction between the aromatic ring structure of tannin and the hydrophobic region of proteins and hydrogen bonds (Goldstein and Swain, 1965; Loomis, 1974; Hagerman and Butler, 1980; Oh *et al.*, 1980). It is, however, believed that phenol–protein complexation is usually the result of the formation of hydrogen bonds and hydrophobic interactions (Hagerman and Butler, 1980), particularly under acidic conditions (McManus *et al.*, 1985).

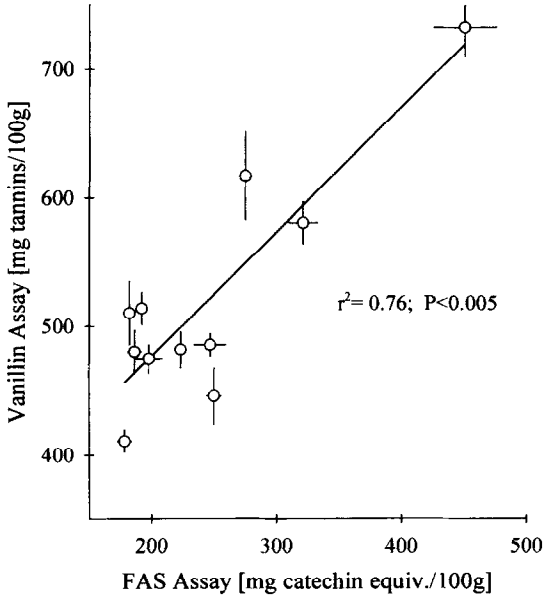


Fig. 5. Linear relationship between the contents of tannin and iron-binding phenolics. (adapted from Naczk *et al.*, 1994).

Kozlowska and Zadernowski (1988) reported that formation of phenol–protein complexes in rapeseed products can be indirectly concluded from the amount of extractable soluble matter in 80% ethanol as this soluble matter is rich in phenolics. They also found that more ethanol-soluble matter was extracted from rapeseed products as the pH of the extraction solution was increased. Zadernowski and Nowak-Polakowska (1992) extracted phenolics of rapeseed with 80% (v/v) methanol and then separated and acidified (pH = 2) aqueous solutions of phenolics into hydrophilic (water-soluble) and lipophilic (diethyl ether-soluble) fractions. They demonstrated, *in vitro*, that the activities of lipase and lipoxygenase were significantly inhibited by the addition of both hydrophilic and lipophilic fractions of rapeseed phenolics. Some results of their study are shown in Table 7. These data indicate that the addition of 0.1–2% of hydrophilic fraction of phenolics brought about a somewhat greater inhibitory effect on lipase activity than that observed for its lipophilic counterpart.

Interactions with proteins: phenolic acids

The presence of phenol–protein complexes in rapeseed meals was demonstrated by subjecting globulin and albumin fractions of rapeseed proteins to alkaline hydrolysis (Zadernowski, 1987). The alkaline hydrolysates contained 6.68 mmol of sinapine acid per g albumin and 0.49 mmol of sinapine acid per g globulin. In addition, Smyk and Drabent (1989) investigated the formation of phenol–protein complexes in model systems consisting of sinapine acid and bovine serum albumin (BSA) by using a fluorescence spectrophotometric

Table 7. Effect of rapeseed phenolics on the in-vitro activity of *Aspergillus oryzae* lipase

	(%) phenolics added	(%) of TGA hydrolysis[a] TAG
Control	0	51
Lipophilic fraction	0.1	48
	1	38
	2	30
Hydrophilic fraction	0.1	45
	1	33
	2	28

[a]Incubation temperature, 42°C; incubation time, 30 min; and pH, 0.5.
Adapted from Zadernowski and Nowak-Polakowska (1992).

technique. These studies indicated that formation of complexes was favoured in neutral and basic pH conditions. Later, Smyk *et al.* (1991) reported that phenolic compound 'X', isolated by Zadernowski (1987), was the predominant phenolic of phenol-albumin complexes. The phenolic compound 'X' is probably a low-molecular weight ester of sinapine acid (Zadernowski, 1987). Using a gel filtration technique, Amarowicz and Kmita-Glazewska (1995) demonstrated that phenolic acids were bound to only selected low molecular weight rapeseed proteins. However, spectroscopic studies indicated that these protein fractions contained only trace amounts of phenolic acids (Amarowicz *et al.*, 1993).

Karamac and Amarowicz (1997) examined the effect of selected phenolic acids on the activity of pancreatic lipase. Hydroxycinnamic acid derivatives exhibited a somewhat stronger inhibition of lipase activity than hydroxybenzoic acid derivatives. They also demonstrated that the inhibitory effect of phenolic acid was influenced by the position of hydroxyl groups as well as the presence of methoxy groups. Salicylic and *o*-coumaric acids exhibited stronger inhibitory effects than corresponding *p*-hydroxybenzoic and *p*-coumaric acids. In contrast, phenolic acids having methoxy groups, such as sinapine and syringic acids, were found to be the weakest inhibitors of lipase activity.

Interactions with proteins: condensed tannins

Leung *et al.* (1979) reported that tannins isolated from rapeseed hulls formed a white precipitate after their addition to a 1% gelatin solution. Later, Mitaru *et al.* (1982) reported that condensed tannins isolated from rapeseed hulls were not capable of inhibiting the activity of α-amylase *in vitro*. In addition, Fenwick *et al.* (1981) and Butler *et al.* (1982) postulated that tainting of eggs may be due to the inhibition of TMA oxidase activity by tannins. This enzyme converts TMA to an odourless and a water-soluble TMA oxide. Later, Fenwick *et al.* (1984) reported that goitrin was a more powerful inhibitor of TMA oxidase than tannins.

Examples of protein-precipitating capacities of condensed tannins isolated from Westar and Cyclone canola hulls as determined by the dye-labelled BSA assay of Asquith and Butler (1985) and the protein precipitation assay of Hagerman and Butler (1978) are given in Table 8. The dye-labelled BSA assay measures the amount of protein precipitated by tannins, whereas protein precipitation assay determines the amount of tannins precipitated by proteins. The data determined at the optimum pHs established by Naczk *et al.* (1996) are similar to those reported by Hagerman and Butler (1978) and Asquith and Butler (1985) for sorghum tannins. Condensed tannins, extracted from canola hull samples, precipitated 23 to 59 mg of BSA g^{-1} hulls. The complete precipitation of BSA from solution corresponds to 80 mg BSA g^{-1} hulls. In contrast, the amount of precipitated condensed tannins expressed as [$A_{510}\,g^{-1}$ hulls] ranged from 1.1 to 4.9. Recently, Naczk *et al.* (1994) suggested characterization of the affinities of canola tannins for proteins by a protein precipitation index calculated using the equation $P_i = B/C$, where B represents the amount of dye-labelled BSA g^{-1} hulls and C represents the content of tannins as determined by the vanillin assay and expressed as mg catechin equivalents per 1 g hulls. They found that the protein precipitation index values for tannins isolated from high-tannin canola hulls (containing 1000 mg or more tannins per 100 g hulls) did not exceed 5.0 mg BSA mg^{-1} of tannins, but for tannins isolated from low-tannin canola hulls (containing less than 500 mg tannins per 100 g hulls) it ranged from 17.7 to 40.7 mg BSA mg^{-1} of tannins. Hagerman and Butler (1978) have demonstrated that protein-precipitating capacity of tannins depends on their molecular weight. Thus, the observed differences in protein precipitation index values may be due to the differences in molecular weights of tannins extracted from low- and high-tannin canola hulls.

Naczk *et al.* (1996) examined the effect of pH and tannin concentration on protein-precipitating capacities of condensed tannins isolated from canola hulls. Figure 6 shows the effect of pH on the amount of tannins precipitating with selected proteins as determined by the protein precipitation assay of Hagerman and Butler (1978). Canola tannins were highly precipitated by unlabelled-BSA fetuin, gelatin and pepsin at pH values between 3.0 and 6.0, but the maximum precipitation by lysozyme occurred at pH > 8.0. The pH optimum for precipitation of unlabelled BSA, fetuin, gelatin, pepsin and lysozyme was found to be 0.3–3.1 pH units below

Table 8. Protein-precipitating capacities of canola tannins as determined by two assays

Sample	Protein precipitation assay ($A_{510}\,g^{-1}$ hulls)	Dye-labelled BSA assay (mg BSA g^{-1} hulls)	Protein precipitation index[a] (mg BSA mg^{-1} tannins)
Westar			
Sample 1	1.1	30.7[a]	17.7
Sample 3	4.0	58.6[a]	3.8
Sample 4	—	23.2[a]	40.7
Cyclone			
Sample 3	4.5	44.2	4.5
Sample 6	4.9	52.2	4.3
Excel	2.0	33.2	23.0

[a]Adapted from Naczk *et al.* (1994).

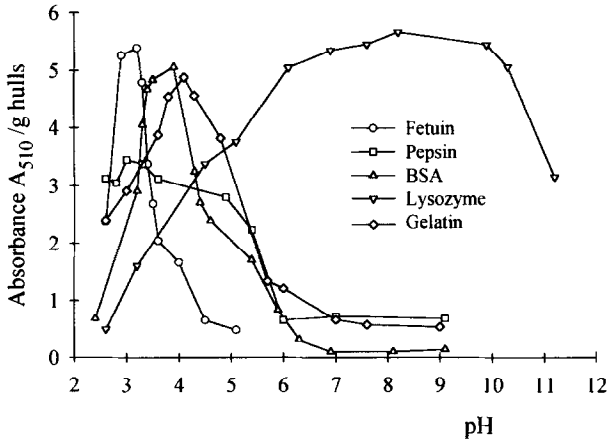


Fig. 6. pH dependence of complex formation between canola tannins and several proteins. (adapted from Naczk *et al.*, 1996).

the isoelectric points of proteins. The results indicate that precipitation of proteins by canola tannins does not depend only on the availability of unionized phenolic groups for hydrogen bonding. A similar effect of pH on the formation of tannin-protein complexes was reported for tannins isolated from sorghum grains (Hagerman and Butler, 1978). The results of this study clearly indicate that pH 4.0 was optimum for carrying out the protein precipitation assay of Hagerman and Butler (1978) for canola tannins and that pH 4.9 (that these authors indicated for sorghum tannins) was not suitable for tannins from canola. Asquith and Butler (1985) developed the dye-labelled BSA method for direct measurement of the amount of protein precipitated by tannins. Naczk *et al.* (1996) found that the dye-labelled BSA was largely precipitated by canola tannins at pH = 3.5 and not 4.8 as indicated for tannins isolated from sorghum grains. They also reported that canola tannins did not show a definitive threshold prior to the binding of dye-labelled BSA but did show a definitive threshold before binding with unlabelled BSA (Fig. 7). The titration curves exhibit a linear relationship between the amount of precipitated tannin-protein complex and the amounts of tested canola tannins added to the system. The observed differences between the titration curves obtained for crude tannins isolated from low- and high-tannin hulls may be due to differences in their affinities for proteins.

EFFECT OF PROCESSING

Processing may affect the sinapine content of rapeseed meals. Larsen *et al.* (1983) have shown that 30 min toasting of dehulled and defatted Erglu rapeseed meal reduced the content of sinapine by 17%. This decrease was ascribed to the formation of oligomeric sinapine derivatives. Later, Jensen *et al.* (1991) reported that heating decreased the content of sinapine and this was accompanied by an increase in the content of lignan-

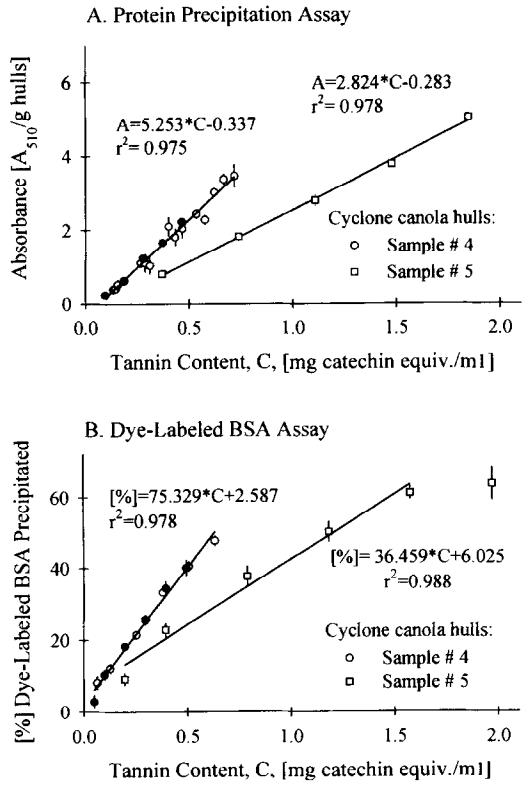


Fig. 7. Titration curves of a known amount of protein with increasing tannin concentration as determined by the protein precipitation assay of Hagerman and Butler (1978) and dye-labelled BSA assay of Asquith and Butler (1985). Dark symbols indicate partially purified tannins; open symbols indicate crude tannins (adapted from Naczk *et al.*, 1996).

type products in rapeseed meal. Recently, Rubino *et al.* (1995, 1996) demonstrated, using model systems, that sinapic acid was readily converted to a lignan, thomasidioic acid, under alkaline conditions. A complete conversion of sinapic acid to thomasidioic acid was attained after incubation of an aerated, aqueous solution of sinapic acid, buffered at pH 8.5, at room temperature (20°C) for 24 h. In contrast, at pH 7, only 30% of sinapic acid was transformed to thomasidioic acid after 24 h. These authors also suggested that additional studies are needed to establish whether a similar reaction will occur when canola meal itself is subjected to alkaline conditions.

Amarowicz *et al.* (1995*a*) reported that moisture contents in seeds (in the range 6.5 to 12.65%) did not affect the total content of phenolics in the cake obtained by pressing of the oil. The total content of phenolics also remained unaffected during a 3-month storage of the cake at room temperature (about 20°C) (Amarowicz *et al.*, 1996).

Processing of rapeseed to protein concentrates using two successive batch extractions (30 min each, 5 l solvent kg^{-1} meal) or counter-current (four-, five- or six-stages) extractions (30 min each stage; 6–10 l solvent kg^{-1} meal) with water, 70% ethanol, acetone-methanol-water (7:7:6, v/v/v), methanol-aqueous ammonia (3.2, v/v) or acidified methanol (pH ranged 2–6.3) reduced the

content of phenolic acids by 60–97% (Dabrowski and Sosulski, 1983). However, Kozlowska and Zadernowski (1983) reported that seven consecutive extraction with 70% ethanol ($10\,l\,kg^{-1}$ meal) was required to reduce the concentrations of phenolics to trace levels.

The content of phenolic compounds in oilseed meals may also be reduced by treatment with ammonia (gaseous or alcoholic solutions). McGregor *et al.* (1983) found that gaseous ammonisation of mustard meal, *Brassica juncea*, removed up to 74% of sinapine. According to Goh *et al.* (1982), extraction of Candle and Tower canola meals by 0.2 M ethanolic ammonia decreased the content of phenolics in the resultant meals by 82 and 39%, respectively; but they did not offer any explanation for the difference in the efficiency of the removal of phenolics from these meals.

A methanol–ammonia–water/hexane extraction system removed 72.4% of the phenolics of canola meals in a laboratory-scale process (Naczk *et al.*, 1986) and up to 80% in a pilot-scale extraction (Diosady *et al.*, 1987). The esterified phenolic acids were extracted more effectively than their free phenolic acid counterparts. The removal of free phenolic acids ranged only from 40.9% for Midas to 75.2%, for Hu You 9, while the amount of esterified phenolics decreased ranged from 82.4% for Midas to 93.1% for Hu You 9. In contrast, 34.6 to 73.6% and 83.8 to 91.4% of the original sinapine acid, in the free and esterified phenolic acids fractions of treated meals, were removed, respectively. However, the insoluble-bound phenolic acids were not extracted to any great extent (Shahidi and Naczk, 1988; Naczk and Shahidi, 1989).

Shahidi and Naczk (1989) found that methanol alone extracted only 16% of tannins present in whole rapeseeds. Addition of 5% (v/v) water to methanol increased the efficiency of tannin extraction to 36%. However, 10% ammonia in 95% methanol–hexane solvent system was most effective for the removal of tannins as the resultant meals contained between 4 and 33% of condensed tannins originally present in the meals. This decrease in tannin content may be due to the extraction of tannins out of the seeds into the polar phase and/or decomposition of tannins to products that were insensitive to vanillin reagent. Gandhi *et al.* (1975) found that ammonia depolymerized the tannins present in salseed meal and the processed meal was nontoxic and palatable. Moreover, tannins, upon alkali treatment, may form phlobaphenes which are both chemically and nutritionally unreactive (Swain, 1979). However, Fenwick *et al.* (1984) reported that treatment of *Brassica napus* meals with ammonia or lime did not affect their tannin content appreciably.

CONCLUSIONS

The predominant phenolic compounds in rapeseed/canola are phenolic acids and condensed tannins. The total content of phenolic acids in rapeseed products may reach 2% and the content of condensed tannins in rapeseed/canola hulls may be over 2%. These high levels of phenolics may be responsible for the dark colour, bitter taste and astringency of rapeseed meal.

The experimental data indicate that both cultivar differences and growing conditions may influence the content of tannins in rapeseed/canola hulls. More detailed research is needed to determine the effect of growing conditions on tannin content of rapeseed hulls.

Phenolic acids and condensed tannins may both form complexes with proteins, thus lowering the nutritional value of rapeseed products. Phenolic acids have a great affinity for the low molecular weight proteins in rapeseed. Inhibition of enzyme activity by phenolic acids is influenced by the position of hydroxyl groups and the presence of methoxyl groups. The protein-precipitating capacity of rapeseed/canola tannins is similar to reported values for tannins isolated from sorghum grains. Further research is still needed to establish the specific mode of interaction of phenolics with proteins in order to develop more efficient processes for production of phenolic-free protein concentrates and isolates from rapeseed meals.

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