Theoretical Analysis of Ultraviolet–Visible Spectra of Various Phenolic Acid Fractions of Canola

David Pink,† Marian Naczk,‡ Katarin Baskin,‡ and Fereidoon Shahidi*,§

Department of Physics and Department of Nutrition and Consumer Studies, St. Francis Xavier University, Antigonish, Nova Scotia B2G 1C0, Canada, and Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9

Spectra of methanolic solutions of free, esterified, and insoluble-bound fractions of phenolic acids isolated from Triton canola were recorded between 250 and 520 nm. These spectra were then analyzed as linear combinations of Gaussian bands using the CHAOS-B (Curve Handling for the Analysis of Overlapping Spectra, version B) computer program. The analysis indicated that between 250 and 520 nm the spectra of the free and esterified fractions were composed of three separate component bands at approximately 280, 300, and 328 nm. The insoluble-bound phenolic acid fraction was composed of four bands at 254, 282, 319, and 384 nm. All three fractions displayed a shorter wavelength (<250 nm) component that could be represented, for $\lambda > 250$ nm, by a Gaussian band located between 217 and 235 nm. The second and fourth theoretical derivative spectra yielded a very good fit to the corresponding spectra obtained from the numerical derivative of the experimental data. The methodology was tested using a model system consisting of mixtures of protocatechuic and sinapic acids. The content of sinapic acid may be estimated with an accuracy of 6%.

INTRODUCTION

Rapeseed is among the world's most important oilseed crops and in Canada is second only to wheat in both value and planted area (Shahidi, 1990). The composition of rapeseed has been significantly altered by Canadian breeders, who have developed canola varieties that contain less than 2% erucic acid and no more than 30 μmol of glucosinolates/g of defatted meal (Daun, 1986), referred to as double zero. Despite the introduction of double-zero rapeseed varieties to common cultivation in many countries and the publication or patenting of a number of methods for the dehulling of rapeseed (Greilsamer, 1983; Kozlowska et al., 1984; Sosulski and Zadernowski, 1981), the use of rapeseed meal as a source of food-grade proteins remains thwarted by the presence of phytic acid and phenolic compounds.

Phenolic compounds of oilseeds are the hydroxylated derivatives of benzoic and cinnamic acids, coumarins, flavonoids, and lignins (Ribereau-Gayon, 1972). Of these, the major phenolic compounds found in rapeseed are in the form of free, esterified, and insoluble-bound phenolic acids (Durkee and Thivierge, 1975; Fenton et al., 1980; Kozlowska et al., 1975, 1983; Krygier et al., 1982a). Phenolic esters and free phenolic acids constitute approximately 80% and up to 16% of the total phenolic compounds of canola, respectively (Krygier et al., 1982a; Naczk et al., 1986). Sinapic acid is the predominant phenolic acid found in rapeseed (Clandinin, 1961). It constitutes over 73% of the free phenolic acids and about 99% of the phenolic acids released from esters and glycosides (Dabrowski and Sosulski, 1984; Krygier et al., 1982a). Minor phenolic acids are *p*-hydroxybenzoic, vanillic, gentisic, protocatechuic, syringic, *p*-coumaric, ferulic, and caffeic acids. In addition, trace amounts of chlorogenic acid are found in the free phenolic acid fraction of rapeseed meals (Kozlowska et al., 1975, 1983; Krygier et al., 1982a; Lo and Hill, 1972).

There is an increasing concern about the nutritional and sensory effects of phenolic compounds in rapeseed since the phenolic content in rapeseed products is much higher than that found in products obtained from other oleaginous seeds (Kozlowska et al., 1990). Phenolic compounds may contribute to the dark color, bitter taste, and astringency of rapeseed meals. They or their oxidized products may also form complexes with essential amino acids, enzymes, and other proteins, thus lowering the nutritional value of rapeseed products. Phenolic compounds have been identified as potent inhibitors of iron absorption, presumably by forming insoluble complexes with iron ions in the gastrointestinal lumen, thus making the iron not available for absorption (Brune et al., 1989). Hence, the presence of phenolic compounds is an important factor in the consideration of rapeseed meal as a protein source in food formulations. However, the available information concerning the undesirable effects of phenolics on the quality of rapeseed meals is still fragmentary (Kozlowska et al., 1983; Sosulski, 1979). There is no reliable method, applicable to all ranges of food products, for the quantification of phenolic compounds. Furthermore, not all phenolics present in foods are of nutritional or sensory concern. Accordingly, the total phenolic content in food cannot be considered as an index of nutritional quality or a sensory characteristic of food products.

The analysis of phenolic compounds is influenced by numerous factors including their chemical nature, extraction methods employed, sample particle size, storage times and conditions, assay procedure employed, and selection of standard as well as the presence of interfering substances such as waxes, fats, terpenes, and chlorophylls. Various methodologies have been developed to assay phenolic compounds, and a number of reviews on the topic have been published (Desphande et al., 1986; Jackman et al., 1987; Makkar, 1989). These assays can be classified as either those which determine total phenolic content or those that measure specific groups or classes of phenolic compounds.

* Author to whom correspondence should be addressed.
† Department of Physics.
‡ Department of Nutrition and Consumer Studies.
§ Department of Biochemistry.

0021-8561/94/1442-1317$04.50/0

A number of spectrophotometric methods have been developed to determine the content of phenolic compounds in plant materials. These assays differ in the principles on which they are based and the various structural groups of phenolic compounds that they determine. One set of these methods is based on a UV spectrophotometric assay since each group of phenolic compounds is characterized by one or several UV absorption maxima. Simple phenolics have absorption maxima in the region between 220 and 280 nm (Owades et al., 1958). However, closely related phenolics display wide variations in their molecular absorptivities. Furthermore, the absorption is affected by the nature of the solvent, the pH, and the possibility of interference by UV-absorbing substances such as proteins, nucleic acids, and amino acids present in the medium. This makes the task of finding a completely satisfactory method quite difficult. The suitability of a UV assay depends, therefore, primarily on the material to be analyzed. For example, the contents of phenolics in tea and beer (Owades et al., 1958), in cereals and legumes (Davis, 1982; Gupta and Haslam, 1980; Sharp et al., 1977), in oilseeds such as canola meals (Blair and Reichert, 1984; Naczk et al., 1992), and in coffee and other beverages (Hoff and Singleton, 1977) have been estimated by UV absorption techniques.

It is advantageous to determine the makeup of the spectral components and functionality of phenolics in foods. Under certain circumstances, spectral bands can be represented as Lorentzian or Gaussian functions [e.g., Pink et al. (1989) and references cited therein], and the analysis of a spectrum as a linear combination of such functions may provide useful information about the components of the system. This work is a first attempt to analyze the UV spectra of various fractions of canola phenolic acids in canola. The objective of this study was to make use of mathematical analysis to separate complex UV spectra of phenolic acids into a combination of spectral bands characteristic of hydroxycinnamic and hydroxybenzoic acid derivatives. The intention is to demonstrate that, by using this procedure, UV–vis spectrometry can be used with confidence to identify and quantify components of rapeseed products even when there is substantial spectral band overlap. Here, the mathematical analysis of spectral bands of canola phenolic acids in the range 250–520 nm will be reported. As a result of this, a more precise description of a spectrum is obtained, and examples will be used to illustrate how such an analysis may serve to identify classes of plenolic components of a sample.

MATERIALS AND METHODS

Isolation of Phenolic Acids. Hexane-extracted meal was prepared by blending 60 g of crushed seeds of Triton canola in a Waring blender with 400 mL of hexanes for 2 min at approximately 15 000 rpm. The meal was then separated by vacuum filtration, and the residual oil was further extracted with hexanes using a Soxhlet apparatus. The resultant defatted meal was dried at 50 °C in a forced-air convection oven.

Two sets of free soluble esters and glycosides and insoluble-bound phenolic acids were isolated from the hexane-extracted Triton meal according to the procedure of Krygier et al. (1982b). The meal (2 g) was extracted six times with a 40-mL mixture of methanol–acetone–water (7:7:6 v/v/v) at room temperature using a Polytron homogenizer (Brinkmann Instruments Ltd., Mississauga, ON) (15 s, 10 000 rpm). After each centrifugation (15 min, 5000 rpm), the supernatants were collected and combined, evaporated at 30 °C under vacuum to approximately 40 mL, and extracted six times with a mixture of diethyl ether–ethyl acetate (1:1 v/v) at a supernatant-to-solvent ratio of 1:1 (v/v). The diethyl ether–ethyl acetate extracts were combined and evaporated to dryness at 30 °C under vacuum. Extracted phenolic acids (referred to as free phenolic acids) were dissolved in methanol. The supernatant, separated earlier and which still contained the esterified phenolic acids, was treated with 30 mL of 4 N NaOH under nitrogen for 4 h at room temperature. The resulting hydrolyzed solution was acidified to pH 2 using 6 N HCl, extracted six times with diethyl ether–ethyl acetate, and evaporated to dryness, as before. The extract of phenolics liberated from their esters was dissolved in methanol. The residual meal after extractions was treated with 20 mL of 4 N NaOH under nitrogen for 4 h at room temperature. The mixture was acidified with 6 N HCl to pH 2 and then centrifuged (15 min, 5000 rpm). The supernatant was extracted six times with diethyl ether–ethyl acetate. The diethyl ether–ethyl acetate extracts were combined and evaporated to dryness at 30 °C under vacuum. Acids liberated from the insoluble residues (referred to as insoluble-bound phenolic acids) were dissolved in methanol.

UV–Visible Spectra of Canola Phenolics. The methanolic solutions of the two sets of free phenolic acids, esterified phenolic acids, and insoluble-bound phenolic acids were diluted with methanol [1:10 (v/v) for insoluble-bound phenolic acids to 1:50 (v/v) for phenolics acids liberated from esters], and their spectra were recorded using a Hewlett-Packard 8452A diode array UV–vis spectrophotometer. Each spectrum was then analyzed to find the number and characteristics of their component Gaussian spectral bands.

Theory and Spectral Analysis. The shape of a spectral band due to a single electronic transition, characterized by a single transition frequency, can become a distribution other than a δ-function when the system being measured is part of a multicomponent system at a finite temperature. In sufficiently simple systems at low temperatures and where the environment in which the molecule finds itself does not apply a substantial perturbation to the molecule, the spectral bands can be represented by Lorentzian functions. At higher temperatures, such as the cases considered here, the band shapes can change since the molecular electronic transitions reflect random fluctuations around an average of the interactions between the molecules and their surroundings. Consequently, the spectrum may be considered, approximately, as a sum of independent variables so that, according to the Central Limit theorem [e.g., Barlett (1980)], it can be represented by the sum of Gaussian functions.

Spectral data of the phenolic acid fractions were analyzed as a linear combination of Gaussian functions

$$A(\lambda) = \sum_n A_n \exp\left[-\frac{(\lambda - \lambda_n)^2}{2\Gamma_n^2}\right] + b_0 + b_1\lambda \qquad (1)$$

where A_n, λ_n, and Γ_n are the absorbance, band center, and bandwidth of the nth component band and $A(\lambda)$ is the total measured absorbance at wavelength λ. The straight line, $b_0 + b_1\lambda$, represents a baseline for which the slope may not be zero. The analysis was performed using the CHAOS-B (Curve Handling for the Analysis of Overlapping Spectra, version B) computer program to analyze spectral absorbance values and their second and fourth derivatives. The original spectrum (zeroth derivative) was analyzed according to eq 1 first. The second derivative of eq 1 was then compared to its numerically differentiated experimental counterpart, and in most cases the two second-derivative spectra agreed satisfactorily. For those cases in which the agreement was not satisfactory, the original (undifferentiated) spectrum was studied to ascertain whether artifacts in the second numerical derivative were present. Such artifacts may arise through noise in the spectrum or by abrupt, possibly noise-related, changes in the absorbance as a function of λ. In all cases disagreement was attributed to such spurious effects. The numerical fourth derivative was found not to be useful since variations in it, due to noise, were of the same magnitude as the apparent fourth derivative of the signal. Numerical fourth derivatives were used, however, to search for gross disagreements with the theoretical spectrum to identify properties of the original spectra. CHAOS-B was written in FORTRAN and was run on a Digital Equipment Corp. DEC5000/200; VOGLE was used for the graphics subroutines. It is not necessary to make use of CHAOS-B to confirm that the original and second- and fourth-

Table 1. Results of the Mathematical Analysis of Spectral Data, Using Gaussian Functions, for Free and Esterified Phenolic Acid Fractions Isolated from Triton Canola

n^a	band center λ (nm)	absorbance A_n	bandwidth Γ_n	rel intensity $[A_n\Gamma_n]$
	Free Phenolic Acids ($b_0 = 0.28$; $b_1 = -0.00056$)			
b	329.0	2.150	18.5	39.78
c	300.5	0.610	14.3	8.72
d	280.5	0.590	21.0	12.39
e	224.0	7.000	14.50	
	Esterified Phenolic Acids ($b_0 = 0.11$; $b_1 = -0.0002$)			
b	327.0	2.570	18.0	46.26
c	299.0	1.190	15.0	17.85
d	273.4	0.600	15.0	9.00
e	217.0	17.500	15.0	
	Insoluble-Bound Phenolic Acids ($b_0 = 0.097$; $b_1 = -0.00011$)			
b	384.0	0.06	38.0	2.28
c	319.0	0.81	25.0	20.25
d	281.7	0.75	14.0	10.05
e	254.2	0.87	14.0	12.18
f	235.0	1.60	9.0	

[a] Band number.

derivative spectra are fit by Gaussian functions with the parameters shown in Table 1. The analyses shown in Figures 1–3 can, in principle, be carried out "by hand".

The intensity, I_n, of the nth band was taken to be the area under it, and this is easily shown to be proportional to $A_n\Gamma_n$ in cases for which $\exp(-\lambda_n^2/2\Gamma_n^2) \ll 1$. This can be seen by making the substitutions

$$x = \lambda/\sqrt{2}\Gamma_n \qquad (2)$$

$$x_n = \lambda_n/\sqrt{2}\Gamma_n$$

so that the intensity becomes

$$\begin{aligned} I_n &= \int_0^\infty A_n \exp\left[-\frac{(\lambda-\lambda_n)^2}{2\Gamma_n^2}\right]d\lambda \\ &= \sqrt{2}A_n\Gamma_n\int_0^\infty \exp[-(x-x_n)^2]dx \\ &\approx \sqrt{2}A_n\Gamma_n\int_{-\infty}^\infty \exp[-(x-x_n)^2]dx \\ &= \sqrt{2}A_n\Gamma_n\int_{-\infty}^\infty \exp[-y^2]dy \end{aligned} \qquad (3)$$

The third step, the extension of the lower limit from 0 to $-\infty$, is justified if $\exp[-x_n^2] \approx 0$, as it is in all cases studied here. The last step, which shows that the integral is independent of x_n when $\exp[-x_n^2] \approx 0$, follows from the substitution $y = x - x_n$. Equation 3 shows that, in such cases, the area under the nth band, I_n, is proportional to $A_n\Gamma_n$. Note that no comment has been made concerning the transition probabilities associated with each component band. Since these are not necessarily equal for two such bands, the areas under the bands will not necessarily represent the relative amounts of each substance present. However, if all transition probabilities are equal, then the areas under each band do represent the relative amount of each substance present.

RESULTS AND DISCUSSION

Figure 1 shows the very good agreement between experimental data and the mathematical analysis of the UV–vis spectra of one of the two sets of methanolic solutions of esterified, free, and insoluble-bound phenolic acids between 250 and 520 nm. The same figure also shows the results of the analysis of the experimental spectra as a linear combination of Gaussian functions, thereby effecting separations of complex spectra into their component bands. It should be clearly understood that

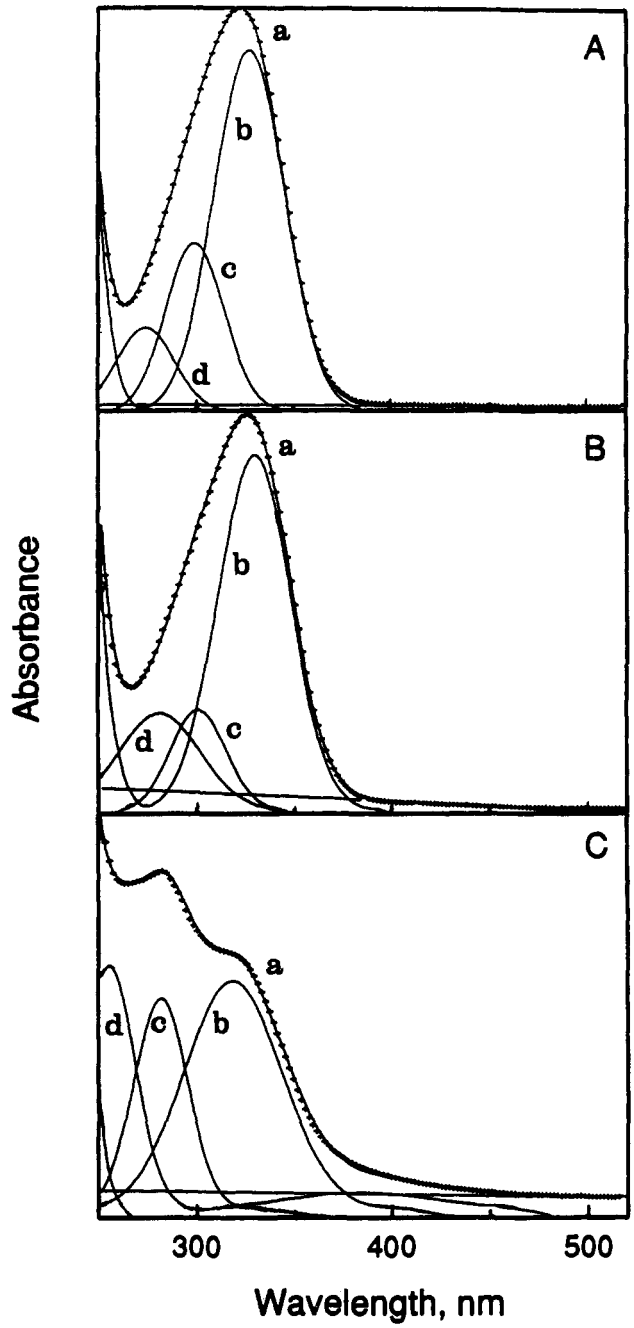

Figure 1. Theoretical analysis of the spectrum of esterified (A), free (B), and insoluble-bound (C) phenolic acids: (+) experimental data. The theoretical spectrum (solid line, a) is composed of Gaussian bands (dashed lines, b–d).

physical significance has been assigned only to those portions of bands lying between 250 and 520 nm. This is especially important in the case of Gaussian functions with band centers at $\lambda < 250$ nm. It is not proposed that there are, necessarily, Gaussian bands in that range but only that their contributions for $\lambda > 250$ nm coincide with Gaussian functions located with band centers at $\lambda < 250$ nm. The band centers, bandwidths, and absorbances used to fit the spectra of phenolic acid fractions in methanol are presented in Table 1. This table also shows the relative intensities of the components. This is given by the areas under the curves, which are proportional to the products $A_n\Gamma_n$ (see above).

Figure 1A shows the experimental and theoretical UV–vis spectrum (250–520 nm) of a methanolic solution of esterified phenolic acids (EPA) liberated from esters and glycosides together with its analysis as a linear combination

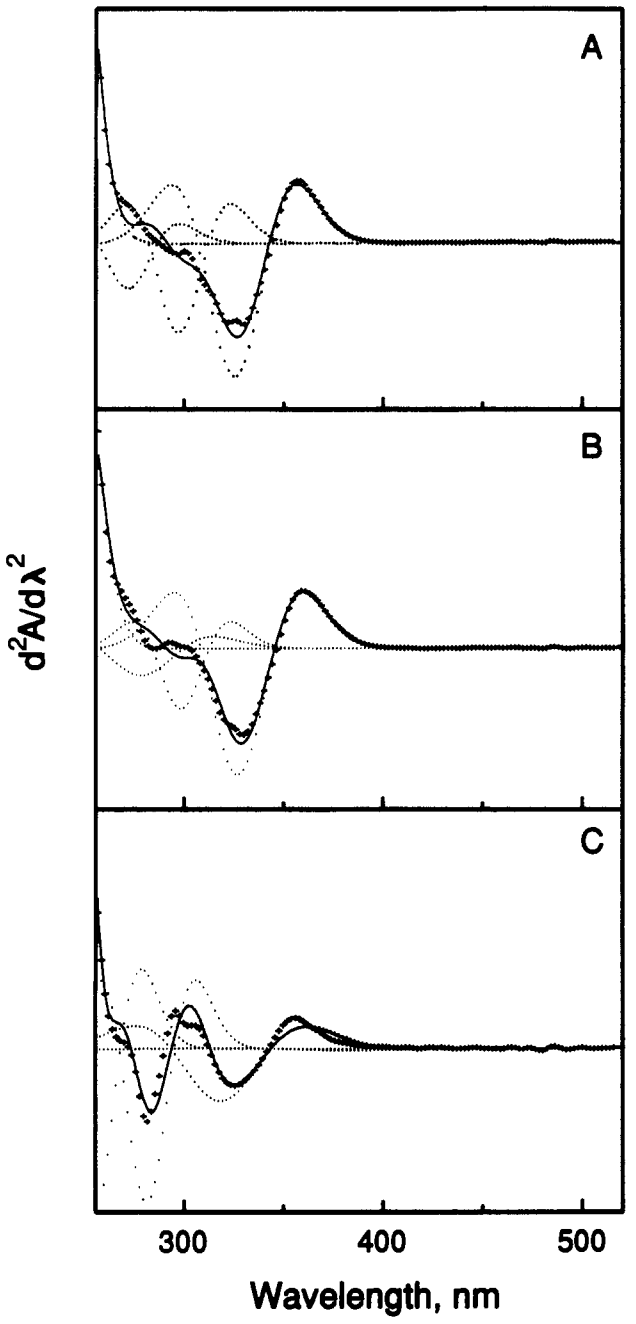

Figure 2. Second derivatives of the spectrum of esterified (A), free (B), and insoluble-bound (C) phenolic acids: derivative of the theoretical spectrum (solid line) and the derivatives of its component Gaussian bands (●); numerical derivative from the experimental data (+).

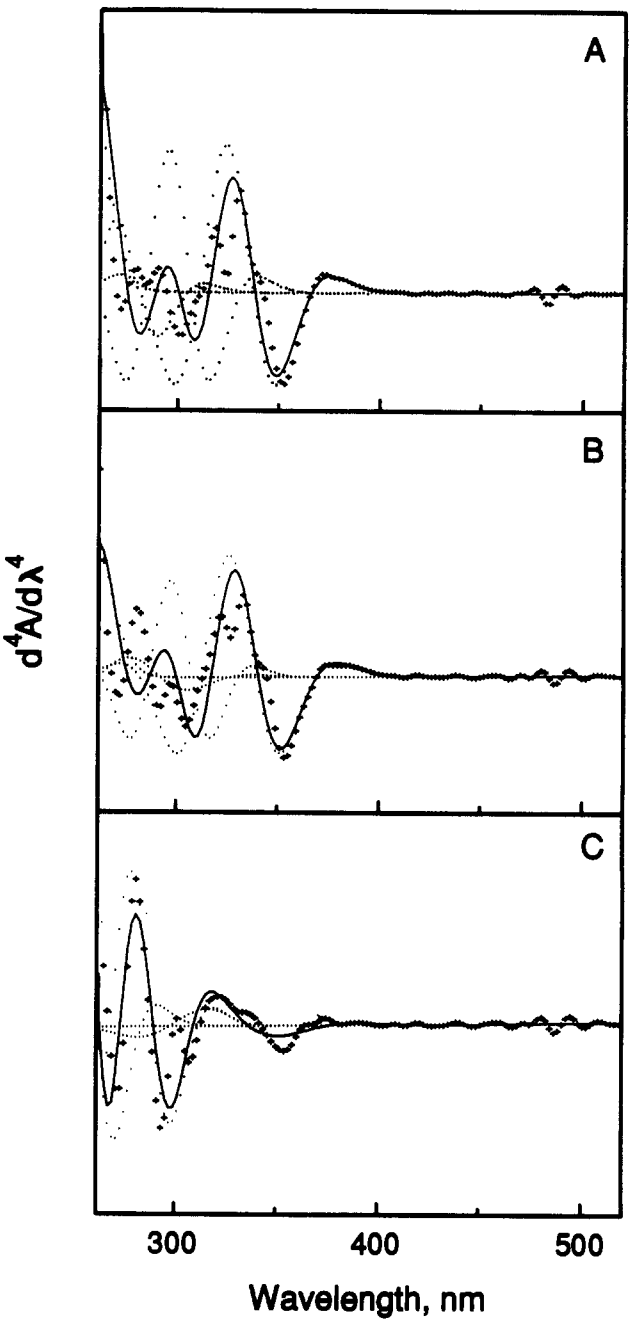

Figure 3. Fourth derivatives of the spectrum of esterified (A), free (B), and insoluble-bound (C) phenolic acids: derivative of the theoretical spectrum (solid line) and the derivatives of its component Gaussian bands (●); numerical derivative from the experimental data (+).

of Gaussian functions as described above. Three bands centered at 273.4, 299.0, and 327.0 nm as well as a component that could be represented by a Gaussian function with $\lambda < 250$ nm and a baseline with negative slope were found to adequately describe the recorded spectrum. The results here show the similarity between the free phenolic acids (FPA) (Figure 1B) and the EPA fractions (Figure 1A). However, the UV spectrum of insoluble-bound phenolic acids (Figure 1C) differs from those obtained for the FPA and EPA fractions. A combination of bands at about 319, 282, and 254 nm together with an effective "band" at $\lambda = 235$ nm and a small band at $\lambda = 384$ nm was deduced for insoluble-bound phenolic acids. The highest intensity band was located at $\lambda = 319$ nm. This may be due to differences inthe composition of phenolic acids in these fractions. The positions of the first three bands are in good agreement with those reported in the literature for benzoic acid derivatives with a maximum absorption at 270–280 nm and with cinnamic acid derivatives with a maximum in the region of 305–330 nm and a shoulder at 290–300 nm (Macheix et al., 1990). The fourth component may be explained by the spectral data reported by Owades et al. (1958), who found that simple phenolics have absorption maxima in the region between 220 and 280 nm. For the reason given above, no significance is given to the value of $A_n\Gamma_n$ for functions that possess band centers located at $\lambda < 250$ nm. In a comparison of the spectra obtained from each of the two duplicate extracts of phenolic acids, it was noted that the component Gaussian bands exhibited shifts of $\leq$1–2 nm.

Figures 2 and 3 show the second and fourth derivatives

Table 2. Results of the Mathematical Analysis of Spectral Data, Using Gaussian Functions, for Sinapic Acid in Mixtures with Protocatechuic Acid

rel concn of protocatechuic acid in mixture (%)	added sinapic acid (μg/mL), A	concn of sinapic acid determined by analysis of spectra (μg/mL), B	$[A - B)/A] \times 100$ (%)
79.4	1.624	1.712	-5.4
49.0	4.060	3.840	5.4
29.2	5.680	5.890	-3.7
19.4	6.496	6.585	-1.4

of the experimental and theoretical spectra for different phenolic acid fractions of canola extracts. Agreement between the experimental and theoretical results is striking when it is realized that the theoretical derivatives are obtained directly from the theoretical absorbance spectra alone and do not make use of further fitting. However, questions may be raised concerning the uniqueness of the analysis carried out here (Jerrard and Pink, 1988, and references cited therein). Therefore, it is necessary that the number of component bands be kept at a minimum and that, for an acceptable analysis with a minimum number of such functions, adequate criteria for acceptability of fit should be set. Due to the existence of noise in the data, some difficulty may be encountered with the analysis of spectra. Accordingly, the use of least-squares methods using restricted sets of data, or the comparison of analytically calculated derivatives of the sum given in eq 1 with numerically calculated derivatives of the experimental data, is important. The first technique ensures that important parts of the spectrum, such as positions and amplitudes of local maxima, are well-fit. The second technique provides separate criteria for fitting not only local maxima and minima but also regions of maximum slope of the data as well as points of inflection. Numerical derivatives of experimental data, however, can incorporate errors which become magnified as higher derivatives are calculated.

The results presented in Table 1 show that the center of the band characteristic of hydroxycinnamic acid derivatives is located between 327 and 329 nm for free and esterified phenolic acid fractions. However, the position of this band center for the insoluble phenolic acid fraction is shifted to 319 nm. The sinapic acid constitutes 65–85% and 71–97% of the free and esterified phenolic acids, respectively, of hexane-extracted meals. On the other hand, the contribution of sinapic acid to the insoluble-bound fraction of phenolic acids varied from 7% to 32% (Naczk et al., 1992). Thus, the contribution from other phenolic acids may be responsible for the shift of the band center of hydroxycinnamic acid derivatives present in the insoluble-bound phenolic acid fraction. Accordingly, it follows that the use of a fixed wavelength for quantification of sinapic acid content by UV methodology may result in significant errors in the estimation of sinapic acid content, particularly for the insoluble-bound fraction of phenolic acids.

The mathematical procedure described here was tested using a model system consisting of mixtures of protocatechuic and sinapic acids. The relative content of protocatechuic acid in these phenolic acid mixtures ranged from 0% to 80%. The results are presented in Table 2 and show that, by using this mathematical approach, the content of sinapic acid in mixture with hydroxybenzoic acid derivative may be under- or overestimated by less than 6%.

CONCLUSIONS

UV spectra have been analyzed as a linear combination of spectral bands to deduce the fractions of free (FPA), esterified (EPA), and insoluble-bound phenolic acids in methanolic solutions isolated from Triton canola. The spectra were analyzed between 250 and 520 nm. The results showed the similarity between the FPA and EPA fractions, with three bands centered at 273, 299, and 327 nm, in good agreement with results reported in the literature. The spectrum of the insoluble-bound fraction exhibited bands centered at 254, 282, 319, and 384 nm. Guidelines to the use of the mathematical techniques described were discussed in relation to work already published. As an example of the accuracy of the method, results were presented for the analysis of samples made up of mixtures of sinapic and protocatechuic acids. It was found that the fractions deduced from the spectral band analysis described here were within 5–6% of the correct values. It is concluded that the use of spectral analysis as described here is a reliable way of identifying and quanitifying the components of rapeseed products even when there is substantial spectral band overlap.

ACKNOWLEDGMENT

The program CHAOS-B was written by Ms. Bonnie Quinn, who is supported by an NSERC Research Grant (to D.A.P.). P.K.J.P.D. Wanasundara (M.U.N.) provided technical assistance. Financial assistance from the Natural Sciences and Engineering Research Council of Canada and the University Council for Research, St. Francis Xavier University, is acknowledged.

LITERATURE CITED

Barlett, M. S. *An Introduction to Stochastic Processes*, 3rd ed.; Cambridge University Press: Cambridge, U.K., 1980.

Blair, R.; Reichert, R. D. Carbohydrates and phenolic constituents in a comprehensive range of rapeseed and canola fractions: nutritional significance for animals. *J. Sci. Food Agric.* **1984**, *35*, 29–35.

Brune, M.; Rossander, L.; Hallberg, L. Iron absorption and phenolic compounds. Importance of different phenolic structures. *Eur. J. Clin. Nutr.* **1989**, *43*, 547–551.

Clandinin, D. R. Effect of sinapin, the bitter substance in rapeseed oil meal, on the growth of chicken. *Poult. Sci.* **1961**, *40*, 484–487.

Dabrowski, K.; Sosulski, F. W. Composition of free and hydrolyzable phenolic acids in defatted flours of ten oilseeds. *J. Agric. Food Chem.* **1984**, *32*, 128–130.

Daun, J. K. Glucosinolate levels in Western Canadian rapeseed and canola. *J. Am. Oil Chem. Soc.* **1986**, *63*, 639–643.

Davis, K. R. Effects of processing on composition and relative nutritive value of green and yellow peas, lentils, and white pea beans. *Cereal Chem.* **1982**, *58*, 454–459.

Desphande, S. S.; Cheryan, M.; Salunkhe, D. K. Tannin analysis of food products. *CRC Crit. Rev. Food Sci. Nutr.* **1986**, *24*, 401–449.

Durkee, A. B.; Thivierge, P. A. Bound phenolic acids of Brassica and Sinapis oilseeds. *J. Food Sci.* **1975**, *40*, 820–822.

Fenton, T. W.; Leung, J.; Clandinin, D. R. Phenolic components of rapeseed meal. *J. Food Sci.* **1980**, *45*, 1702–1705.

Greilsamer, B. Dehulling and extraction of rapeseed. In *Proceedings of the Sixth International Rapeseed Conference*; Paris, 1983; Vol. 2, pp 1496–1501.

Gupta, R. K.; Haslam, E. Vegetable tannins: structure and biosynthesis. In *Polyphenols in Cereals and Legumes*; Hulse, J. H., Ed.; International Development Research Centre: Ottawa, Canada, 1980; pp 15–25.

Hoff, J. F.; Singleton, K. I. A method for determination of tannins in foods by means of immobilized enzymes. *J. Food Sci.* **1977**, *42*, 1566–1569.

Jackman, R. L.; Yada, R. Y.; Tung, M. A. A review: separation and chemical properties of anthocyanins used for their

qualitative and quantitative analysis. *J. Food Biochem.* **1987**, *11*, 279–308.

Jerrard, R. J.; Pink, D. A. Peak Locator for Multicomponent Spectra. *Biochim. Biophys. Acta* **1988**, *996*, 415–418.

Kozlowska, H.; Sabir, M. A., Sosulski, F. W.; Coxworth, E. Phenolic constituents of rapeseed flour. *Can. Inst. Food Sci. Technol. J.* **1975**, *8*, 160–163.

Kozlowska, H.; Rotkiewicz, D. A.; Zadernowski, R.; Sosulski, F. W. Phenolic acids in rapeseed and mustard. *J. Am. Oil Chem. Soc.* **1983**, *60*, 1119–1123.

Kozlowska, H.; Zadernowski, R.; Lossow, B. Pol. Pat. 37490, 1984.

Kozlowska, H.; Naczk, M.; Shahidi, F.; Zadernowski, R. Phenolic Acids and Tannins in Rapeseed and Canola. In *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*; Shahidi, F., Ed.; Van Nostrand Reinhold: New York, 1990; pp 193–210.

Krygier, K.; Sosulski, F. W.; Hogge L. Free, esterified, and insoluble-bound phenolic acids in rapeseed flours and hulls. *J. Agric. Food Chem.* **1982a**, *30*, 334–336.

Krygier, K.; Sosulski, F. W.; Hogge, L. Free, esterified, and insoluble-bound phenolic acids. Extraction and purification. *J. Agric. Food Chem.* **1982b**, *30*, 330–333.

Lo, M. T.; Hill, D. C. Composition of aqueous extracts of rapeseed meals. *J. Sci. Food Agric.* **1972**, *23*, 823–830.

Macheix, J.-J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990; pp 1–98.

Makkar, H. P. S. Protein precipitation methods for quantification of tannins: a review. *J. Agric. Food Chem.* **1989**, *37*, 1197–1202.

Naczk, M.; Diosady, L. L.; Rubin, L. J. The phytate and complex phenol content of meals produced by alkanol-ammonia/hexane extraction of canola. *Lebensm. Wiss. Technol.* **1986**, *19*, 13–16.

Naczk, M.; Wanasundara, P. K. J.P.D.; Shahidi, F. Facile spectrophotometric quantification method of sinapic acid in hexane-extracted and methanol-ammonia-water-treated mustard and rapeseed meals. *J. Agric. Food Chem.* **1992**, *40*, 444–448.

Owades, J. L.; Rubin, G.; Brenner, M. W. Determination of food tannins by ultraviolet spectrophotometry. *J. Agric. Food Chem.* **1958**, *6*, 44–47.

Pink, D. A.; Hamboyan, L.; Aboud, H. Theoretical Analysis of Ultraviolet Spectroscopic Studies of the Feathering of Cream in Coffee. *J. Dairy Res.* **1989**, *65*, 749–754.

Ribereau-Gayon, P. *Plant Phenolics*; Oliver and Boyd: Edinburgh, 1972.

Shahidi, F. Rapeseed and canola: global production and distribution. In *Canola and Rapeseed: Production, Chemistry, Nutrition, and Processing Technology*; Shahidi, F., Ed.; Van Nostrand Reinhold: New York, 1990; pp 3–13.

Sharp, R. N.; Sharp, C. Q.; Kattan, A. A. Tannin content of sorghum grain by UV spectrophotometry. *Cereal Chem.* **1977**, *55*, 117–118.

Sosulski, F. W. Organoleptic and nutritional effects of phenolic: review. *J. Am. Oil Chem. Soc.* **1979**, *56*, 711–715.

Sosulski, F. W.; Zadernowski, R. Fractionation of rapeseed meal into flour and hull component. *J. Am. Oil Chem. Soc.* **1981**, *58*, 96–98.

Received for review January 24, 1994. Accepted March 22, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, May 1, 1994.