Multivariate Model for the Prediction of Total Phenolic Acids in Crude Extracts of Polyphenols from Canola and Rapeseed Meals: A Preliminary Study

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ABSTRACT: The feasibility of using UV spectrophotometry to develop multivariate models for prediction of total phenolic acids content in crude polyphenol extracts from defatted canola and rapeseed meals was investigated. The polyphenols were extracted from the meals with methanol/acetone/water (7:7:6, by vol). Partial least squares regression was used to correlate the spectral data of the crude polyphenols in methanol between 320 and 355 nm with the total phenolic acid content in canola and rapeseed meals. The Folin–Denis assay was used to provide reference data for creating the model. The predictive ability of the model is good, as indicated by the RPD value (the ratio of the SD of data to the standard error of calibration) of 3.84.

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KEY WORDS: Canola, meal, partial least squares (PLS), phenolic acids, rapeseed, UV spectroscopy.

Phenolic compounds of oilseeds are hydroxylated derivatives of benzoic and cinnamic acids, coumarins, flavonoids, and lignins (1). Of these, the major phenolic compounds found in rapeseed and canola products are phenolic acids (2) and soluble and insoluble condensed tannins (3,4).

The total content of phenolic acids in rapeseed and canola meals is up to five times higher than that found in soybean meals, and 10–30 times more phenolic acids are present in rapeseed and canola flours than in flours obtained from other oleaginous seeds. The rapeseed phenolic acids are derivatives of benzoic and cinnamic acids and are present in rapeseed and canola in the free, esterified, and insoluble-bound form. Free phenolic acids constitute about 15% of the total phenolic acids in rapeseed and canola meal, whereas esterified phenolic acids constitute up to 80% of total phenolic acids. Free and esterified phenolic acids are considered to be the major contributors to objectionable taste in rapeseed and canola proteinaceous products. Sinapic acid is the predominant phenolic acid, and it constitutes up to 90% of the free phenolic acids and from 70.9 to 96.7% of phenolic acids liberated from esters (5).

The analysis of polyphenols is affected by their chemical nature, the extraction procedure employed, and the assay employed for their quantification. Numerous spectrophotometric assays have been proposed for assaying polyphenols in plant material. One group of methods is based on UV spectrophotometric assays in which each class of phenolic compounds is characterized by one or more UV absorption maxima. However, closely related phenolic substances may display wide variations in their molar absorptivities. In addition, the absorption is affected by the solvent, the pH, and the presence of interfering UV-absorbing substances such as nucleic and amino acids (6,7). UV spectroscopic assays have been proposed for the estimation of polyphenol content in tea and beer (8), cereals and legumes (9), and oilseeds (10,11).

This study examined whether it is possible to predict the total content of phenolic acid (free + liberated from their esters) in canola and rapeseed meals from the UV spectra of crude polyphenol extracts in methanol, using the partial least squares (PLS) regression method, in order to simplify future analyses.

MATERIALS AND METHODS

Sample preparation. Seeds of Alto, Bounty, Celebra, Colt, Cyclone, Delta, Eclipse, Excel, Horizon, Legend, Parkland, Profit, Tobin, Tristar, Triton Trumph, Westar, and Windfield canola varieties and Leo, Mar, Marita, and Polo Polish rapeseed varieties (41 samples total) were ground and defatted with hexane for 12 h using a Soxhlet apparatus and then dried at room temperature. The canola seeds were grown at several locations in western Canada in 1991–1998. The samples of Polish rapeseed varieties were grown in central Poland in 1995.

Preparation of crude phenolic extracts. The soluble crude polyphenols were isolated from hexane-extracted meals as described by Krygier *et al.* (2). The meal (1.0 g) was extracted six times with a 20 mL mixture of acetone/methanol/water (7:7:6, by vol) at room temperature using a Polytron homogenizer (Brinkman PT 3000; Littau, Switzerland) (60 s, 15,000 rpm). After each centrifugation (10 min at maximum speed, 1750 × *g*) using an IEC Clinical Centrifuge (International Equipment Co., a Division of Damon, Needham Heights, MA), the supernatants were collected, combined, and evaporated to near dryness at 40°C under vacuum. This residue was dissolved in 25 mL of methanol and centrifuged again as described above. The methanolic solution of crude polyphenols is referred to as Extract A.

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Isolation of phenolic acids. Ten milliliters of Extract A was evaporated to near dryness at 40°C under vacuum, and the residue was suspended in 20 mL of triply distilled water and treated with 30 mL of 4 M NaOH under nitrogen for 4 h at room temperature. The resulting hydrolyzed solution was acidified to pH 2 with 6 M HCl and extracted six times with diethyl ether (1:1, vol/vol). The diethyl ether extracts were combined and evaporated to dryness as described above. The residue, containing phenolic acids, both free and liberated from their esters, was dissolved in methanol. The methanolic solution of phenolic acids is referred to as Extract B.

Quantification of phenolics. The total content of phenolic compounds in Extracts A and B was estimated using the Folin–Denis assay (7). The total phenolic content, *C*, in milligrams *trans*-sinapic acid equivalent per 100 g oil-free meal, was calculated using the equation:

$$C = k(0.1721A_{725} - 0.0124) \quad [1]$$

where correlation coefficient $r^2 = 0.993$. Here k is the dilution factor, ranging from 3000 to 10,000, and A_{725} is the absorbance value at 725 nm.

Spectral measurements. The spectra of Extracts A were recorded using a Beckman 7400 diode array UV-vis spectrophotometer. For the spectral analysis, the Extracts A were diluted with methanol at a ratio of 1:20 (vol/vol), and then spectra were collected from 240 to 400 nm at 1.25 nm resolution at room temperature (path length 1 cm). Pure methanol was used as the blank.

Data treatments. Mathematical analysis of the data was performed using PLSplus/IQ v. 3.0 software (Galactic Industries Corp., Salem, NH). The PLS method was used to develop the multivariate calibration model. The statistical analysis of the data (linear regression, *t*-test, standardized residuals, standard errors of estimates) was carried out using the SigmaStat v. 2.03 (SPSS, Chicago, IL) software package.

RESULTS AND DISCUSSION

The range of total polyphenol concentration in the canola and rapeseed meals used in this study lay between 857 and 2,357 and that of total phenolic acids between 751 and 2,070 mg sinapic acid equivalents per 100 g sample, respectively. These results are in good agreement with those published (5).

Figure 1 shows UV spectra of methanolic solutions of both crude phenolics (Extract A) and total phenolic acids (free + liberated from their esters) (Extract B) isolated from Profit canola meal. These UV spectra show one absorption maximum related to the presence of the nontannin fraction of canola and rapeseed meal polyphenols (11). This absorption maximum may be due to the presence of phenolic acids, notably hydroxycinnamic acid derivatives (6,12).

The UV absorption of crude extracts of plant polyphenols is affected not only by their composition, the nature of the solvent, and pH of solution but also by the presence of interfering substances such as proteins, amino acids, and nucleic acids (6,7). Therefore, the use of traditional UV spectroscopic assays tends to lead to an overestimation of the polyphenol content of crude extracts from plant materials. This makes the task of finding a satisfactory UV spectrophotometric assay quite cumbersome. However, these problems can be overcome by employing the PLS method (13,14).

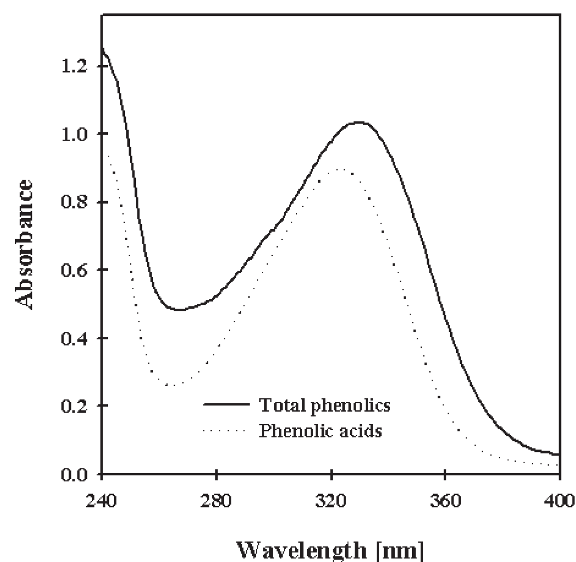

FIG. 1. UV spectra of methanolic solutions of crude phenolics and total phenolic acids isolated from Profit canola meal.

The use of the PLS method for the development of multivariate calibration models to establish correlations between IR spectral data and chemical food quality indices has been reviewed by van de Voort (15). Although the PLS method is more commonly used in the IR and near-IR spectral range (16,17), it can also be employed in the UV-vis range (11,18).

The database consisted of 41 samples of meals of several canola and rapeseed varieties. The Folin–Denis assay was used to generate reference data, the chemical indices of this work. The total phenolic acid content was expressed in sinapic acid equivalents, because sinapic acid is the predominant phenolic acid in canola and rapeseed meals. In the spectral range 240–400 nm, a narrower region, 320–355 nm, was selected to develop and then cross-validate the PLS calibration model. This spectral region has been identified in the literature as characteristic of methanolic solutions of hydroxycinnamic acid derivatives (6,7). Sinapic acid is the predominant hydroxycinnamic acid derivative found in rapeseed and canola meals. It constitutes 65–85 and 70.9–96.7% of the free and esterified phenolic acids, respectively (5,7,10). The optimal number of factors required to develop a satisfactory PLS calibration model was determined on the basis of the prediction residual error sum of squares (13) and was found to be six. The data set used in this study was small; therefore, it was necessary to employ the entire set to develop the PLS calibration model. According to Williams and Sobering (19), setting aside 8–10 samples for a validation set, in case of a small database (40 samples), may lead to misleading results, whereas cross-validation statistics would provide a better rep-

resentation of the true efficiency of the model. Therefore, the PLS model was tested only by the cross-validation procedure involving the rotation of one sample out at each prediction (20).

The results of cross-validation predictions are plotted in Figure 2 in terms of predicted vs. actual total phenolic acid contents measured by the Folin–Denis assay. The *t*-test was used to test the null hypothesis that the intercept and slope values of the linear regression (equation is given in Fig. 2) are zero. The results of this analysis indicated that the slope was different from zero ($P < 0.001$) but that the intercept was not ($P > 0.05$). About 95% of the standardized residuals, used here as regression diagnostics, were between −2 and +2, indicating that points on the graphs were not far from the regression lines (21). Standard errors of the intercept (SE_{int}) and slope (SE_{slope}) are given on the graph.

The statistics of the cross-validation test, expressed as the squared coefficient of correlation (r^2) between the spectrally predicted and chemically determined values, are given in Table 1. The RPD value, calculated as the ratio of the SD of the data to the standard error of calibration (SEC), was 3.84. According to Williams (22) and Sinnaeve *et al.* (23), an RPD value of 3–5 indicates that the model is suitable for screening—in this case, for total phenolic acid content in canola and rapeseed meals. The percentage relative error of calibration calculated as the ratio of SEC to the mean of phenolic content was 6.1%. This suggests that the Folin–Denis assay provides good reference data for the PLS calibration model.

In this study, the PLS method was used to develop a multivariate calibration model correlating the UV spectral data of methanolic solutions of crude polyphenolic extracts (Extract A) with the total phenolic acid contents in meals (Extract B)

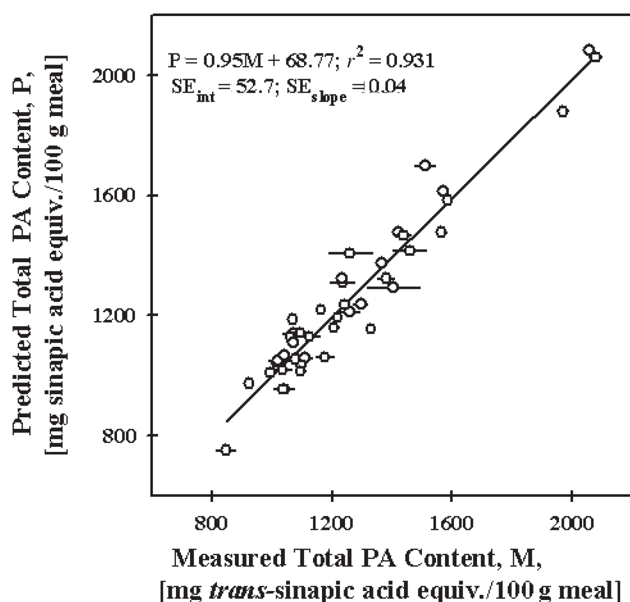

FIG. 2. Plot of total phenolic acid (PA) content predicted by the partial least squares model vs. phenolic acid content measured by the Folin–Denis assay as shown by the cross-validation results. SE_{int}, standard error of intercept; SE_{slope}, standard error of slope.

TABLE 1
Statistics of the Cross-Validation Predictions of the PLS Model

PA[a] range[b]	Mean PA content[b]	SD	r^2	SEP[c]	RPD[d]
751–2082	1276.6	294.8	0.932	76.7	3.84

[a]PA, phenolic acids.
[b]mg sinapic acid equivalents per 100 g meal.
[c]SEP, standard error of performance.
[d]RPD = SD/SEP.

as determined by the Folin–Denis assay. The results show that UV spectroscopy can be used in combination with the PLS method for the quantitative prediction of phenolic acid content in crude polyphenolic extracts (Extract A) from either canola or rapeseed meals, within the concentration range used for the calibration. The best correlation was obtained in the 320–355 nm region, which includes the absorption maximum specific for canola and rapeseed phenolic acids in methanol. This study further suggests that if the calibration set can be increased to represent a greater variety of meals, then a more accurate prediction of phenolic acid content will be possible by simply recording the UV spectra of crude extracts of phenolic acids in methanol and applying our calibration model. A possible use of this procedure is for the screening of plant materials as potential sources of natural antioxidants and in plant breeding for selecting low-phenolic-acid varieties for the production, for example, of protein concentrates and isolates.

Because the UV-based multivariate model reduces the use of chemicals, it is ecologically sounder than the method commonly used for the quantification of phenolic acids in crude extracts of canola and rapeseed meals. This novel approach also shortens the time required to determine the total content of phenolic acids. At least 6 h are needed to determine the phenolic acid content in the crude extract (Extract A) by the method of Krygier *et al.* (2), but only about 10 min by using the multivariate model procedure described here.

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