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Total Phenolics, Condensed Tannins, and Protein-Precipitable Phenolics in Young and Mature Leaves of Oak Species

Rajinder K. Dawra,* Harinder Paul S. Makkar, and Bhupinder Singh

Total phenolics, condensed tannins, proanthocyanidins, leucoanthocyanins, and protein-precipitable phenolics were determined in young and mature leaves of *Quercus incana*, *Quercus semecarpifolia*, and *Quercus ilex*. Total phenols in young leaves of both *Q. semecarpifolia* and *Q. incana* were higher, but no difference was observed for *Q. ilex*. Condensed tannins increased with maturity except in the case of *Q. ilex* where no change was observed; the same trend was observed for proanthocyanidins and leucoanthocyanins. Protein-precipitable phenolics and total phenols were highest in *Q. semecarpifolia* and lowest in *Q. ilex*. The protein-precipitable phenolics had an excellent correlation ($r = 0.995$) with total phenols.

The utilization of tree foilage as feed for ruminants is a common practice during periods of fodder shortages (Negi et al., 1979). Oak leaves are used as fodder for ruminants in India and various other developing countries (Neser et al., 1982; Makkar et al., 1986). Incorporation of oak (*Quercus incana*) leaves into mixed rations up to a level of 14% has been reported to be safe (Negi et al., 1979; Lohan et al., 1983). Feeding at higher levels or given as the sole feed results in oak poisoning (Makkar et al., 1986). Adverse effects of oak leaves to ruminants have been attributed to the presence of tannins in the leaves (Lohan et al., 1983; Makkar et al., 1986). The deleterious effects of tannins depend on the quantity, types, and their protein-precipitating capacity (Feeny and Bostock 1968; Feeny, 1970; Makkar et al., 1987). In the present study, total tannin content as total phenolics, condensed tannins, proanthocyanidins, leucoanthocyanins, and protein-precipitable phenolics of young and mature leaves of three oak species, *Q. incana*, *Quercus semecarpifolia* and *Quercus ilex*, is presented. The implication of the results in relation to adverse effects of oak leaves in livestock is discussed.

Indian Veterinary Research Institute, Regional Station, Palampur (H.P.) 176 061, India.

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Table I. Total Phenols, Condensed Tannins, and Protein-Precipitable Phenolics in Young and Mature Leaves of Three Oak Species

	Q. incana		Q. semecarpifolia		Q. ilex	
	young	mature	young	mature	young	mature
total phenols,[a] mg TAE/g dry wt	81.5	66.3	337.7	137.1	31.0	30.7
condensed tannins, mg catechin equiv/g dry wt	8.1	41.3	8.1	67.1	2.2	2.2
proanthocyanidins,[b] mg QBE/g dry wt	5.7	117.0	8.8	167.4	4.7	3.4
leucoanthocyanins, A_{550nm}/g dry wt	22.0	130.1	4.9	240.5	3.2	3.3
protein-precipitable phenolics, mg TAE/g dry wt	21.8	27.2	208.0	83.2	2.6	2.6

[a] TAE = tannic acid equivalent. [b] QBE = bisulfited quebracho equivalent.

MATERIALS AND METHODS

Q. incana, *Q. semecarpifolia*, and *Q. ilex* young (about 1 month old) and mature leaves (about 10 months old) were collected from each marked tree of different species in the vicinity of the Regional Station. These were dried at 50 °C and stored at −20 °C in sealed polyethylene bags until further use. Leaves were powdered in the laboratory grinding mill and sieved through a 1-mm screen before analysis.

Leaf powder (60 mg) was extracted twice for 8 min with 4 mL of 50% (v/v) aqueous methanol. The extraction was carried in a test tube covered wtih a marble using a heating block maintained at 95 °C (Martin and Martin, 1982). The final volume was adjusted to 10 mL with 50% aqueous methanol.

Extracts were kept on ice and used the same day. Total phenolics were estimated as described by Julkunen-Tiitto (1985). Condensed tannins were determined by the vanillin hydrochloride method of Broadhurst and Jones (1978). Proanthocyanidins were determined as described by Martin and Martin (1982). The method of Bate-Smith (1981) was used for determining leucoanthocyanins. The protein (bovine serum albumin) precipitating capacity, measured as protein-precipitable phenolics, was determined as described by Hagerman and Butler (1978). The results were expressed as tannic acid equivalents (TAE). To determine protein-precipitable phenolics, the extracts were concentrated 10 times first by removal of methanol in vacuo at 37 °C followed by freeze-drying and proper aliquots were taken. The results were obtained from regression coefficients (slopes) of linear regression fitted to measurements performed at at least four different concentrations on three separate extracts as suggested by Martin and Martin (1982).

RESULTS AND DISCUSSION

Total phenols, condensed tannins, proanthocyanidins, leucoanthocyanins, and protein-precipitable phenolics in young and mature leaves of *Q. incana*, *Q. semecarpifolia*, and *Q. ilex* are given in Table I. Total phenols were highest in *Q. semecarpifolia* at both stages studied. In the cases of *Q. incana* and *Q. semecarpifolia* total phenolic content was higher in young leaves relative to that in mature leaves, whereas in *Q. ilex* no difference was observed in young and mature leaves. A decrease in the content of phenols with maturation has also been reported in *Quercus gambelii* and *Quercus havardii* by Nastis and Malechek (1981) and Dollahite et al. (1966), respectively. However, for *Quercus robur*, Fenny (1970) reported a higher tannin content in mature leaves. The difference could be attributed to species variation. This is further supported by the observation in *Q. ilex* where no difference in tannin was observed in young and mature leaves (Table I).

Condensed tannin levels were lower in the young leaves compared to those in mature leaves of *Q. incana* and *Q. semecarpifolia*, while no difference was observed in young and mature leaves of *Q. ilex*. The same trend was observed for proanthocyanidins and leucoanthocyanins (Table I). Feeny and Bostock (1968) have also observed an increase in the content of condensed tannins in the leaves of *Q. robur* with maturation. Protein-precipitable phenolics/protein-precipitating capacity is taken as a measure of the antinutritional properties of a tannin or a group of tannins (Martin and Martin, 1982). In the present study a good correlation ($r = 0.995$) was observed between total phenolic content and protein-precipitable phenolics. For *Q. incana* total phenols and protein-precipitable phenolics do not follow the same trend. This could be due to the different nature of phenolics bound to protein, as the values obtained by the method used depend not only upon the amount of phenolics but also upon its structure (Martin and Martin, 1982). The condensed tannins did not correlate well ($r = 0.109$) with protein-precipitable phenolics, suggesting that condensed tannins do not contribute appreciably to the protein-precipitation capacity. The hydrolyzable tannins and other simple phenols appear to have a higher effect on protein-precipitation capacity, and the former group of phenolic compounds in oak leaves could be responsible for the antinutritional effects of oak leaves.

Higher contents of total phenolics and protein-precipitation capacity in young leaves of *Q. incana* and *Q. semecarpifolia* indicate that young leaves are more toxic than the mature leaves. Further, the leaves of *Q. semecarpifolia* could be expected to be most harmful, followed by *Q. incana* and *Q. ilex*. The chemical composition and digestibility values are available only for *Q. incana* (Negi et al., 1979), the most widely available oak species in the region (Makkar et al., 1986). There is a need to study other oak species for their nutritive values. The tannin contents of different oak species along with their nutritive values would be helpful in selecting species for plantation under social forestry programs.

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Carbon-13 Nuclear Magnetic Resonance for the Qualitative and Quantitative Analysis of Structurally Similar Disaccharides

Nicholas H. Low, Tom Brisbane, Glen Bigam, and Peter Sporns*

The use of carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy for both the qualitative and quantitative analyses of structurally similar glucose–glucose and glucose–fructose disaccharides in honey was investigated. Two of the main problems associated with the quantitative use of ^{13}C NMR, which are differences in carbon relaxation times and the variable nuclear Overhauser effect (NOE), were overcome by employing a relaxing reagent (chromium acetylacetonate). ^{13}C NMR was applied to the analysis of the complex mixture of minor disaccharides found in honey.

Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy has been used extensively as a method for the structural determination of carbohydrates. A number of recent reviews have been written on the application of this methodology to the study of carbohydrates both in solution and in the solid state (Bock and Thoegersen, 1982; Coxon, 1980; Gorin, 1981; Inch, 1972; Pfeffer, 1984; Vliegenthart et al., 1983).

Coxon (1980) and Rathbone (1985) have recognized that ^{13}C NMR spectroscopy could be used for the quantitative analysis of carbohydrates. Some of the problems associated with the use of ^{13}C NMR for quantitation of carbohydrates have been documented by Wehrli and Wirthlin (1976). These problems include differences in carbon relaxation times (T_1 and T_2) and nuclear Overhauser effects (NOE), viscosity effects, temperature effects, solubility, digital resolution, and the low sensitivity of the ^{13}C nuclei. It has been recognized that the most serious of these effects are due to the long relaxation times and the variable nuclear Overhauser effects (Wehrli and Wirthlin, 1976; Levy and Nelson, 1972). Berry et al. (1977) and Czarniecki and Thornton (1977) have shown that the NOE for the carbon nuclei in carbohydrates vary dramatically, which introduces errors into quantitative measurements. Coxon (1980) has also found that the T_1 for most carbon nuclei in carbohydrates is less than 1 s. In order for carbon nuclei to relax completely after a 90° pulse it is necessary to wait a period of at least $5T_1$ (or 5–6 s) before another pulse is applied.

Departments of Food Science (P.S.) and Chemistry (T.B., G.B.), University of Alberta, Edmonton, Alberta T6G 2P5, Canada, and Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada (N.H.L.).

Cerbulis et al. (1978) noted that, in order to quantitate carbohydrates by ^{13}C NMR, the NOE needed to be suppressed. This may be accomplished by employing the gate-decoupling technique (Freeman et al., 1972), which requires turning off the decoupler for long periods (or 5–7 T_1) between data acquisition if a 90° pulse is used.

Due to the inherent lack of sensitivity of ^{13}C nuclei to NMR detection when compared to ^{1}H nuclei (approximately 6000 times less sensitive), either concentrated carbohydrate solutions or long accumulation times are required to obtain reasonable spectra. Use of the gated-decoupling technique or waiting $5–6T_1$'s makes the acquisition time necessary to obtain a ^{13}C spectrum lengthy and expensive. In addition, the use of concentrated carbohydrate solutions may be very difficult or impossible with rare or expensive carbohydrates.

Applications of ^{13}C NMR to the quantitation of carbohydrates in food are few. Blunt and Munro (1976) applied ^{13}C NMR to the quantitation of carbohydrates extracted from various tissues of *Pinus radiata*. When relaxation delays of $>4T_1$ (>4 s) were employed, the levels of fructose, glucose, and sucrose were determined with standard deviations ranging from 3 to 8%. Tamate and Bradbury (1985) applied ^{13}C NMR spectroscopy to the analysis of carbohydrates in tropical root crops. Comparisons of the results obtained by ^{13}C NMR to those obtained by HPLC showed deviations of 3–30%.

In this work we investigated the use of ^{13}C NMR spectroscopy for the qualitative and quantitative analyses of the oligosaccharides found in honey. ^{13}C NMR analyses were carried out on a Bruker 400-MHz spectrometer operating in the Fourier transform (FT) mode. These analyses were performed on standard solutions of reduced disaccharides prepared by weighing a fixed amount of the reduced disaccharide and dissolving it in 0.5 mL of DMSO-d_6 with the addition of a fixed concentrations of

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