Estimation of phenolic acids in cinnamon, clove, cardamom, nutmeg and mace by high performance liquid chromatography

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ABSTRACT

Phenolic acids of five commercially important spices, namely, cinnamon, clove, cardamom, nutmeg and mace were quantitated by high performance liquid chromatography using external standard method. Protocatechuic acid was the predominant phenolic acid in cinnamon bark, while gallic acid dominated in clove buds. The major phenolic acids in cardamom seeds and nutmeg were caffeic acid + vanillic acid while ferulic acid and synapic acid predominated in mace.

Key words : cardamom, cinnamon, clove, mace, nutmeg, phenolic acids, spices.

Abbreviations

GLC : Gas liquid chromatography

HPLC : High performance liquid chromatography

Introduction

Cinnamon (*Cinnamomum verum* Bercht Presl.), clove (*Syzygium aromaticum* (L.) Merril & Perry), cardamom (*Elettaria cardamomum* Maton), nutmeg and mace (*Myristica fragrans* Houtt.) are important spices of commerce, valued for their flavouring properties. Phenolic compounds though present as minor components in these spices are known to be responsible for their astringency and bitterness (Singleton & Esau 1969). Among the phenolics identified so far, phenolic acids are the most common and widely distributed. These compounds are known to impart bitter taste (Spanos & Wrolstad 1992) and possess important pharmacological properties (Harborne & Baxter 1993). It was therefore of interest to separate and quantitate the phenolic acids in the title spices under study.

Two classes of naturally occuring phenolic acids, namely, hydroxy cinnamic and benzoic acids generally occur either as glycosides or as quinic acid deriva-

tives. Several methods for the analysis of these glycosides using GLC and HPLC are reported in literature (Moller & Herrmann 1982; Winter & Herrmann 1984; Klick & Herrmann 1988). An HPLC method for the analysis of phenolic acid composition in green pepper berries was also recently reported (Variyar & Bandyopadhyay 1994). Schulz & Herrmann (1980) have quantified the phenolic acid content of several spices including the title spices of different origin using GLC. However, there is a lack of information on the composition of phenolic acids in spices of Indian origin.

Despite GLC being a sensitive method for analysis of phenolic acids and their glycosides, the limited stability and low volatility of phenolic acids necessitated purification and derivatization prior to analysis. HPLC, on the other hand, is a milder and rapid technique for the analysis of the above class of phenolic compounds. The present paper, therefore, attempts to separate and quantify the phenolic acid constituents as their aglycone in commercial samples of Indian cinnamon, clove, cardamom, nutmeg and mace using HPLC.

Materials and methods

HPLC analysis was carried out on a Pharmacia LKB HPLC system equipped with model 2248 pumps, a LCC-2252 gradient controller, a VWM-2141 double wavelength UV detector set at 292 nm and a Rheodyne model 7125 injector with 200 µl sample loop. Peaks were registered on a REC-1 recorder. All solvents (analytical reagent grade) were from E. Merck (India) Ltd., Bombay, India and were redistilled before use. HPLC grade methanol, water and acetic acid were passed through 0.45 µl filters from Millipore Filter Corporation, USA, prior to analysis. Standard phenolic acids, namely, gallic, p-coumaric, caffeic, ferulic, synapic, syringic, p-hydroxy benzoic, protocatechuic, vanillic, gentisic and salicylic acids were obtained from Sigma Chemical Company (USA) and used directly.

Commercial samples of cinnamon, clove, cardamom, nutmeg and mace were procured from local markets. A sample of 25 g each was separately ground to a fine powder in a grinder for 1-2 min. The powdered sample was then individually extracted with 80% aqueous methanol (7 × 100 ml). The extracts were separately pooled, evaporated to dryness under vacuum and then dissolved in distilled water to make a 10% (w/v) solution in all cases. Each of the above solution was washed with peroxide free diethylether and then separately subjected to acid hydrolysis (1M HCl, 1h, 100°C). The hydrolyzed solution was extracted with peroxide free diethylether (5 × 30 ml). The organic layers in each case were separately pooled, washed free of acid, dried over sodium sulfate and then evaporated to dryness by a slow stream of nitrogen. The residue was made to 0.01% solution in methanol and directly used for chromatographic analysis.

Qualitative analysis

Qualitative analysis was carried out on a Mino RPC S 5/20, C_2/C_{18} analytical column (Pharmacia LKB Biotechnology Uppsala, Sweden) 200 × 4.6 mm ID. The samples were eluted with 2% acetic acid as solvent A and methanol as solvent B using linear gradient elution from 0% to 100% B in A over a period of 30 min, at a flowrate of 1.2 ml/min (Variyar & Bandyopadhyay 1994). Peaks were identified by comparing their retention time with that of authentic standards in-

jected separately under the same condition. The composition of the separated components was determined from peak areas measured from peak-height times peak-width at half height.

Quantitative analysis

Quantitative analysis using the external standard method was attempted to quantify phenolic acid constituents in the ether solubles obtained from the above hydrolyzed spice samples. The phenolic acids present were estimated from the standard curves using protocatechuic acid, gallic and ferulic acid as standards in case of cinnamon, clove and mace, respectively, and caffeic acid as standard in case of cardamom and nutmeg. One mg of each of the above standards was separately dissolved in 10 ml distilled water to obtain 0.01% standard solution. Aliquots of these solutions ranging in concentration from 0.1 - 15 µg were injected under the same experimental conditions as above. A plot of amount of standard vs. peak area was used to obtain standard curves. To quantify the phenolic acids in the samples, 50-100 µl of each of the above other solubles were analyzed under identical conditions as described above. The amounts of phenolic acid in the individual spice samples were calculated from the standard curves and expressed in ppm.

Results and discussion

A HPLC chromatogram of phenolic acid fraction isolated from cinnamon bark and clove buds is presented in Fig.1. Phenolic acid mixture from other samples also gave similar qualitative separation on HPLC. The chromatographic profile of phenolic acids were similar to that reported by Seo & Morr (1984).

The two groups of phenolic acids, namely, hydroxy cinnamic acids and benzoic acids exhibited different absorption maxima. For example, hydroxy cinnamic acids gave an absorption maxima at 330 nm while hydroxy

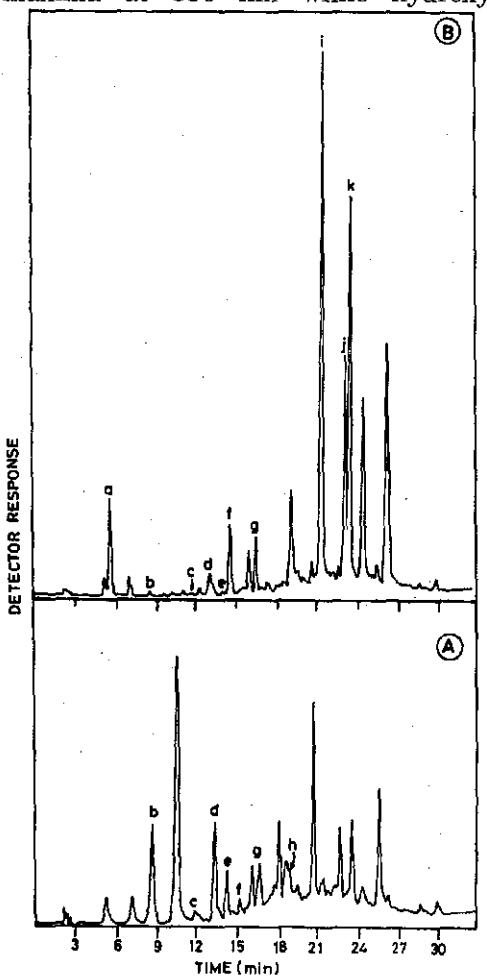

Fig.1. HPLC chromatogram of ether solubles obtained from A. cinnamon bark and B. clove buds

a. gallic acid b. protocatechuic acid c. gentisic acid and p-hydroxy benzoic acid d. caffeic acid and vanillic acid e. syringic acid f. p-coumaric acid g. ferulic aid and synapic acid h. salicylic acid i. quercertin j. eugenol k. kampferol

benzoic acids had their absorption maxima at 250-260 nm. A compromise value at 292 nm was therefore selected that was a mean of these two absorption maxima. Most of the phenolic acids showed a reasonably high absorbance value at this wave length.

The quantitative distribution of phenolic acids in the five spices under study is summarized in Table 1. The present HPLC method was unable to separate gentisic acid from p-hydroxy benzoic acid, vanillic acid from caffeic acid and ferulic acid from synapic acid. Thus concentrations of these phenolic acids were reported as their respective sums.

Cinnamon

Protocatechuic acid was the major phenolic acid in the cinnamon bark sample. The content of this acid was lower in the present study accounting for only 24% of the total phenolic acids compared to 55.8% and 40.3% as reported by Schulz & Herrmann (1980) in Sri Lankan and Chinese cinnamons, respectively. The content of gentisic acid was comparable with that of the Sri Lankan sample while Chinese cinnamon was reported (Schulz & Herrmann 1980) to be devoid of it. In contrast, a high amount of ferulic + synapic acid observed in the present study compares well with that of the total content of these two acids in Chinese cinnamon (Schulz & Herrmann 1980) (Fig. 1). Thus a wide variation in phenolic acid content occurs between different samples based on their geographical locations.

Clove

The phenolic acids identified were qualitatively similar to that reported by Schulz & Herrmann (1980). Gallic acid, the major phenolic acid identified, constituted only 28% of the total phenolic acid content compared to their very high value (93%) reported earlier (Schulz & Herrmann 1980). This discrepancy could be accounted for by the oxidative loss of gallic acid during acid hydrolysis and their lower solubility in either soluble fraction analyzed by HPLC in the present study. The contents of protocatechuic acids were very low as reported earlier (Schulz & Herrmann 1980) (Fig.1).

Cardamom

The distribution of phenolic acids in cardamom seed showed wide quantitative differences with the values reported in literature (Schulz & Herrmann 1980). For example, while ferulic acid was reported to be the major phenolic acid contributing to 81% of the total phenolic acids, it accounted for only 4.8% in the present study. In contrast, vanillic and caffeic acid together accounted for 68% of the phenolic acid unlike the reported value of 5.1%. The content of gentisic, p-coumaric, syringic and salicylic acids were, however, comparable with the reported values with the latter two acids being present only in trace amounts. These variations could probably be dependent on the intrinsic characteristics of the sample, maturity, geographical location and agroclimatic conditions under which they were grown.

Nutmeg and mace

Wide differences in phenolic acid contents were also noted in nutmeg and mace compared to that reported in literature (Schulz & Herrmann 1980). Ferulic and synapic acids together accounted for 16.5% and 32.1% of the total phenolic acid content of nutmeg and mace, respectively. In contrast, the

Table 1. Quantitation of phenolic acid constituents of dry cinnamon, clove, cardamom, nutmeg and mace by HPLC

Sample	Phenolic acid (ppm)							
	Gallic acid	Protocate-chuic acid	Gentisic acid + p-hydroxy-benzoic acid	Caffeic acid + vanillic acid	Syringic acid	p-coumaric acid	Ferulic acid + Synapic acid	Salicylic acid
[1]Cinnamon	Absent	27.4	11.10	19.80	10.30	11.00	24.00	12.2
[2]Clove	174.7	10.0	7.50	99.80	7.50	169.70	129.80	Trace
[3]Cardamom	Absent	0.1	0.50	1.85	Trace	0.15	0.13	Trace
[3]Nutmeg	Absent	0.2	0.69	1.30	0.37	0.29	0.57	Trace
[4]Mace	Absent	Trace	Trace	1.60	1.60	1.40	2.60	0.8

Each value is an average of two determinations

Standard used : [1]Protocatechuic acid; [2]Gallic acid; [3]Caffeic acid; [4]Ferulic acid

values reported in literature were 80% and 65%, respectively. Synapic acid was reported by Schulz & Herrmann (1980) to be the major phenolic acid of nutmeg and mace. Similar results were also observed in case of mace samples in the present study. These variations in phenolic acid content could probably arise due to various factors as indicated in case of cardamom.

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