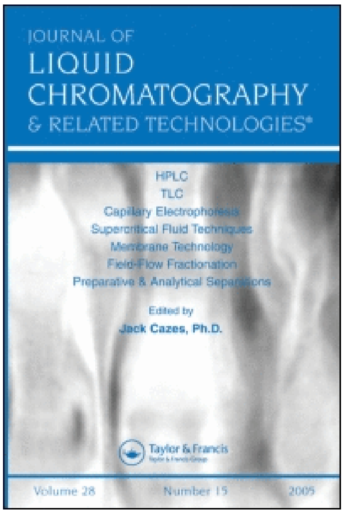

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC Analysis of Nonvolatile Flavor Components in Tamarind (*Tamarindus Indica L.*)

Amrik L. Khurana[a]; Chi-Tang Ho[b]
[a] Whatman, Inc., Clifton, New Jersey [b] Department of Food Science, Cook College New Jersey Agricultural Experiment Station Rutgers, the State University of New Jersey, New Brunswick, New Jersey

To cite this Article Khurana, Amrik L. and Ho, Chi-Tang(1989) 'HPLC Analysis of Nonvolatile Flavor Components in Tamarind (*Tamarindus Indica L.*)', Journal of Liquid Chromatography & Related Technologies, 12: 3, 419 — 430
To link to this Article: DOI: 10.1080/01483918908051744
URL: http://dx.doi.org/10.1080/01483918908051744

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(3), 419-430 (1989)

HPLC ANALYSIS OF NONVOLATILE FLAVOR COMPONENTS IN TAMARIND (TAMARINDUS INDICA L.)

AMRIK L. KHURANA[1] AND CHI-TANG HO[2]
[1]*Whatman, Inc.*
9 Bridewell Place
Clifton, New Jersey 07014
[2]*Department of Food Science*
Cook College
New Jersey Agricultural Experiment Station
Rutgers, the State University of New Jersey
New Brunswick, New Jersey 08903

ABSTRACT

Nonvolatile flavor components in the pulp of Tamarindus Indica L. fruit have been identified and analyzed by using high performance liquid chromatography. Presence of total sugars, individual amino acids and organic acids was determined on PartiSphere-5 NH_2 column. Various sugar components were resolved on PartiSphere-5 PAC column. Resolution of typical organic acids and amino acids was achieved on PartiSphere-5 WCX column.

INTRODUCTION

Tamarind is the fruit of the tree Tamarindus Indica L. It has been found to be an excellent laxative and is used in tropical countries as a diuretic remedy for bilious disorders, jaundice and catarrh. A cooling medicinal drink made of this fruit has been proved to be beneficial in fever in many parts of India (4). The beverages containing Tamarind were found to be beneficial towards Escherichia coli, Bacillus subtilis, Salmonella Typhi and Klebsiella Pneumonia (1). The petroleum ether extract of the plant was found to possess antifungal activities. Tamarind seed gum has been used to manufacture heat stable flavors (5). The fruit pulp of Tamarind is being used as a sweet and sour sauce through out India. A chip dip containing sour cream base with a blend of fruit, tamarind, flavors and spices have been developed by Saratoga Specialties (2).

Isolation, identification and analysis of the nonvolatile components of the tamarind fruit pulp is limited to the use of thin layer chromatography (3). Presence of sugars such as glucose, mannose and maltose and acids like serine, proline, pipcolinic acid, citric acid, oxalic acid and succinic acid have been reported by using TLC of the methylated extract of Tamarind. The present investigations were conducted to analyze the complex mixture of nonvolatile components by using HPLC.

EXPERIMENTAL

Materials

Glucose, fructose, rhamnose, alanine, serine, leucine, proline and phenylalanine were purchased from Sigma Chemical Company (St. Louis, MO). Oxalic acid, citric acid, succinic acid, tartaric acid, pipcolinic acid, ascorbic acid, potassium phosphate, sodium phosphate, caffeine, ammonium hydroxide, acetonitrile and methanol were bought from Aldrich Chemical Co. Inc. (Milwaukee, WI).

Packing Material

PartiSphere-5 PAC, PartiSphere-5 NH_2 and PartiSphere-5 WCX columns were obtained from Whatman Inc. (Clifton NJ).

Sample Preparation

The extracts of tamarind fruit pulp was prepared as follows: 1 gram of the pulp was dispersed in 5 ml of water, stirred for 15 minutes and then centrifuged. Solutions of the internal standards were prepared by dissolving 50 mg of ascorbic acid in 1 ml of water, 50 mg of caffeine in 1 ml of water and 120 mg of rhamnose in 1 ml of water.

HPLC Analysis

HPLC was performed using RI detector, Differential Refractometer R 401 (Waters Associate, Inc., Milford, MA), a programmable solvent delivery system, Series 3B (Perkin-Elmer Corp., Norwalk, Conn.); a manual injection valve, with 50 ul loop (Valco Instruments Co., Houston, TX) and a chart recorder (Laboratory Data Control, Riviera Beach, FL).

Water containing 0.01 M KH_2PO_4 was used as a mobile phase to analyze various components from tamarind fruit pulp solution on Whatman PartiSphere-5 NH_2 column. Ascorbic acid served as an internal standard. A mixture of water and acetonitrile (90:10, v/v) was used as a mobile phase to resolve components such as alanine from serine, tartaric acid from phenylalanine and proline from leucine on PartiSphere-5 WCX column. Caffeine was used as an internal standard in these cases (Fig. 2, 3 and 6). Resolution of oxalic acid from glucose and fructose mixture was achieved on PartiSphere-5 PAC column by using aqueous solution of 0.01 M KH_2PO_4 (Fig. 4) as a mobile phase. Weight against area calibration curves was used to quantify oxalic acid in the mixture. Water and acetonitrile mixture (20:80, v/v) was used as a mobile phase to resolve glucose from fructose on PartiSphere-5 PAC column (Fig. 5). Rhamnose served as an internal standard to quantify glucose and fructose. Authentic samples of glucose, fructose, alanine, serine, leucine, proline, phenylalanine, pipcolinic acid, oxalic acid, citric acid, succinic acid and tartaric acid were used to analyze these components on various columns.

RESULTS AND DISCUSSION

High performance liquid chromatography has been used to analyze various nonvolatile components from tamarind fruit pulp solution. A partial resolution of sugars, amino acids and organic acids was achieved on PartiSphere-5 NH_2 column (Figure 1). The components were quantified by using ascorbic acid as an internal standard. Glucose, fructose and oxalic acid coeluted on this column. Similarly, the amino acids like alanine, phenylalanine and leucine did not resolve from serine, tartaric acid and proline respectively. The unresolved components were resolved, identified and quantified by using PartiSphere-5 PAC and PartiSphere-5 WCX columns (Figures 2-6). The unresolved mixture of alanine and serine was collected from PartiSphere-5 NH_2 column and resolved on PartiSphere-5 WCX by using caffeine as an internal standard (Figure 2). Similarly tartaric acid-phenylalanine and leucine-proline mixtures were collected from PartiSphere-5 NH_2 column and resolved on PartiSphere-5 WCX column. The components were quantified by using caffeine as an internal standard (Figures 3 and 6). PartiSphere-5 PAC column was used to resolve oxalic acid from sugars in the mixture as collected from PartiSphere-5 NH_2 column (Figure 4). The sugar mixture containing glucose and fructose was collected from PartiSphere-5 PAC column

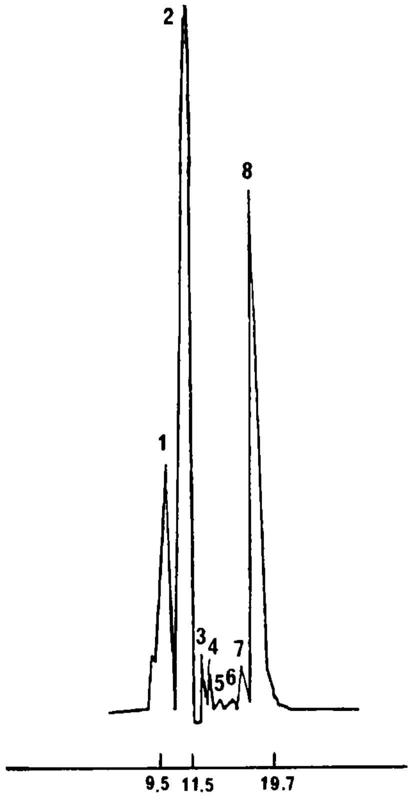

Fig. 1. 1(alanine and serine, 23%), 2(glucose, fructose and oxalic acid, 57%), 3(leucine and proline, 6%), 4(phenylalanine and tartaric acid, 4.7%), 5(pipcolinic acid, 1.7%), 6(succinic acid, 1%), 7(citric acid, 6%), 8(ascorbic acid as an internal standard). Mobile phase: water containing 0.01M KH_2PO_4; Flow rate: 0.2 ml/min; Detector: RI; Sample volume injected: 30 ul + 30 ul of internal standard (50 mg/1 ml); Sample description: Tamarind pulp solution (1g/5ml water); Column: Whatman Partisphere-5 NH_2, 25 cm x 4.6 mm (I.D.).

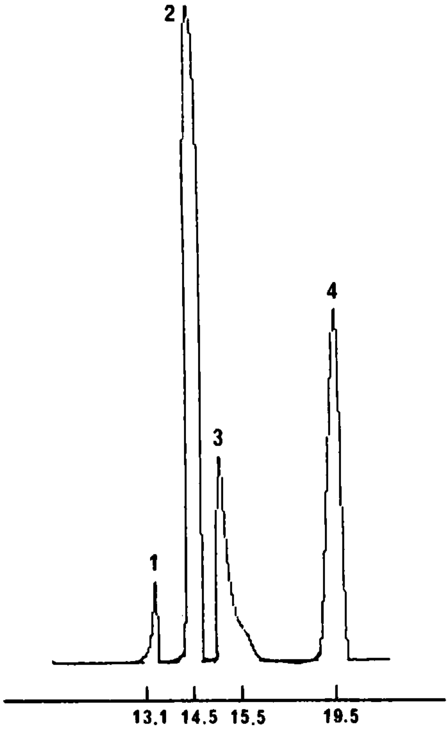

Fig. 2. 1(void volume), 2(alanine, 60%), 3(serine, 37.5%), 4(caffeine as an internal standard). Mobile phase: water : acetonitrile (90:10, v/v); Flow rate: 0.2 ml/min; Detector: RI; Sample description: mixture containing alanine and serine as collected from Whatman PartiSphere-5 NH_2 column (Figure 1); Column: Whatman Partisphere-5 WCX, 25 cm x 4.6 mm (I.D.).

(Figure 4) and resolved on the same column by using water : acetonitrile (20:80, v/v) mixture containing 1% NH_4OH as a mobile phase (Figure 5).

The PartiSphere-5 NH_2 column (Figure 1) resolved various tamarind mixture components by mixed mechanisms such as cation-exchange and reverse phase. KH_2PO_4 was

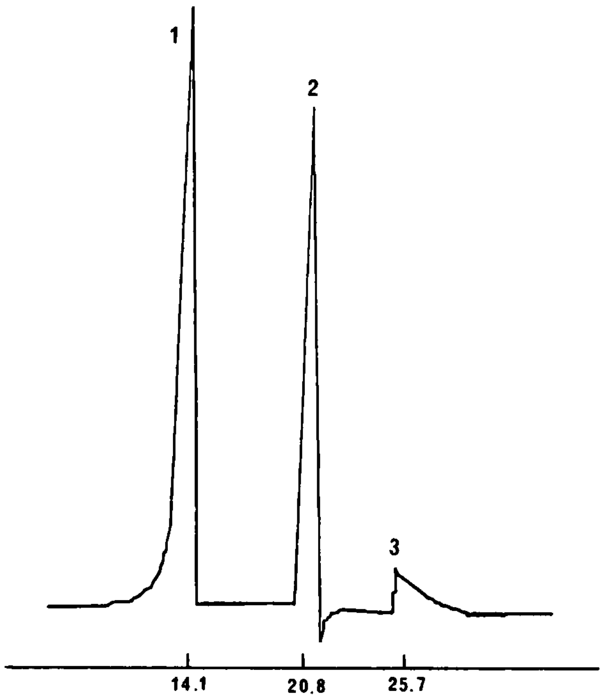

Fig. 3. 1(tartaric acid, 65%), 2((caffeine as an internal standard), 3(phenylalanine, 35%). Mobile phase: water : acetonitrile (90:10, v/v); Flow rate: 0.2 ml/min; Detector: RI; Sample description: mixture containing tartaric acid and phenylalanine as collected from Whatman PartiSphere-5 NH_2 column (Figure 1); Column: Whatman Partisphere-5 WCX, 25 cm x 4.6 mm. (I.D.).

used in the mobile phase to provide counter-ion effect. Resolution of various amino acids on the PartiSphere-5 WCX column was achieved by reverse phase mechanism. PartiSphere-5 PAC column has a cyano end group and also an amino group in the side chain of the bonded phase. Separation of sugar mixture of glucose and fructose from oxalic acid on this column occurred due to a

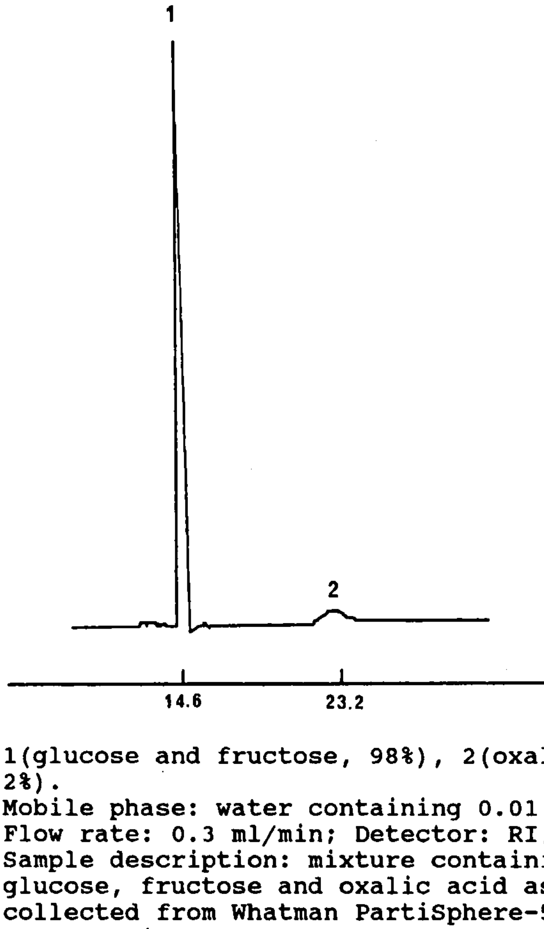

Fig. 4. 1(glucose and fructose, 98%), 2(oxalic acid, 2%).
Mobile phase: water containing 0.01 M KH_2PO_4; Flow rate: 0.3 ml/min; Detector: RI; Sample description: mixture containing glucose, fructose and oxalic acid as collected from Whatman PartiSphere-5 NH_2 column (Figure 1); Column: Whatman Partisphere-5 PAC, 25 cm x 4.6 mm. (I.D.).

cation-exchange mechanism. KH_2PO_4 was used to provide a counter-ion effect (Figure 4). It was found necessary to exclude KH_2PO_4 and include 1% NH_4OH in the mobile phase to resolve glucose from fructose on PartiSphere-5 PAC column.

Figure 1 shows the composition of the resolved as well as unresolved components in the fruit pulp of

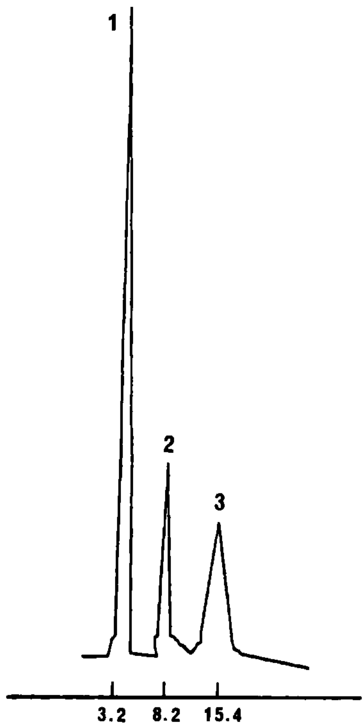

Fig. 5. 1(glucose, 67%), 2(rhamnose as an internal standard), 3(fructose, 33%). Mobile phase: water : acetonitrile (20:80, v/v) containing 1% NH_4OH; Flow rate: 1 ml/min; Detector: RI; Sample description: mixture containing glucose and fructose as collected from Whatman PartiSphere-5 PAC column (Figure 4); Column: Whatman Partisphere-5 PAC, 25 cm x 4.6 mm. (I.D.).

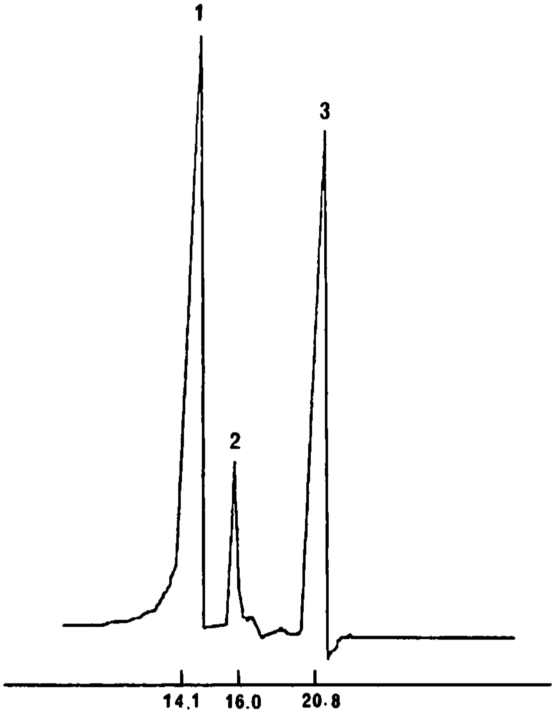

Fig. 6. 1(proline, 90%), 2(leucine, 10%), 3(caffeine as an internal standard).
Mobile phase: water : acetonitrile (90:10, v/v); Flow rate: 0.2 ml/min; Detector: RI; Sample description: mixture containing proline and leucine as collected from Whatman PartiSphere-5 NH_2 column (Figure 1); Column: Whatman Partisphere-5 WCX, 25 cm x 4.6 mm. (I.D.).

tamarind. The ratios of the various unresolved components can be deduced from the data given in the Figures 2-5. The percentages of individual components in the mixture are presented in the Table 1.

TABLE 1. Percentages of the Various Components in the Tamarind Pulp Extract

Components	Percentages
Alanine	14.2
Serine	8.8
Glucose	37.5
Fructose	18.4
Oxalic Acid	1.1
Leucine	0.6
Proline	5.4
Phenylalanine	1.7
Tartaric Acid	3.1
Pipcolinic Acid	1.7
Succinic Acid	1.6
Citric Acid	6.0

ACKNOWLEDGEMENT

New Jersey Agricultural Experiment Station Publication No. D-10205-5-88 supported by State Funds and Hatch Regional Project NE-116.

REFERENCES

1. Alian, A., El-Ashwah, E. and Eid, N. J. Food Sci., 11, 109 (1983).

2. Banner, R. J., Ingredient Strategies. Toms River, NJ, 3 (1987).

3. Hasan, S. K., Salam, A. and Wahid, M. A. Pak. J. Sci., 30, 71 (1978).

4. Kadans, J. M. Encyclopedia of Fruits, Vegetables, Nuts and Seeds. Parker Publishing Co., Inc., West Nyack, NY, 169 (1973).

5. Tadao, K., Ichiro, M. and Toshihiko, Y. Jpn. Kokai Tokkyo Koho, 61, 56056 (1986).

6. Tressl, R. and Drawert, F. J. Agric. Food Chem., 21, 560 (1973).