



Research Article

ESTIMATION OF VITAMINS, MINERALS AND AMINO ACIDS IN PALMYRA PALM

(*Borossus flabellifer* L.) FRUIT PULP

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ABSTRACT

The present investigation was carried out to determine the presence of vitamins, minerals and amino acids in palmyra palm fruit pulp. HPLC technique has been used for the determination of amino acids and vitamins. Minerals were estimated by AOAC methods. The results indicated that the content of fat soluble vitamins D3 (0.970 µg), vit E (0.020µg) and K (0.004µg) and the Vitamin A (retinol acetate) was found below detectable level. Thiamine was 0.523 µg whereas riboflavin, pyridoxine and niacin were 9 µg, 3.9 µg and 0.5 µg respectively. Ascorbic acid was estimated by 2, 6-dichlorophenol indophenol (DCPIP) titration method and its content was 16.9 mg/100gm. The pulp contains high amount of sodium (20mg/100gm) followed by Phosphorus (15 mg/100gm), magnesium (10.2 mg/100g), copper (10mg/100g) and calcium (8.76 mg/100 g). Some of EAA s and NEAA are present in the pulp.

Key words: Palmyra fruit pulp, vitamins, minerals, amino acids

INTRODUCTION

Everything we eat directly or indirectly obtain from plants. Plants provide the basic diet for over 5 billion people in our world today¹. Palmyra palm or Asian toddy palm is botanically known as *Borossus flabellifer* L., belongs to the family *Arecaceae*². It is commonly cultivated in India, Southeast Asia, Malaysia, and occasionally in other warm regions including Hawaii and southern florida³. It has numerous medicinal uses for all parts of the palmyra palm. The pulp of the mature fruit relieves dermatitis. Its fruit is a kind of drupe, large, and fibrous with usually 3 to 5 nut like portions, each of which encloses a seed when it is tender. From female palms only the economic edible products obtained are immature endosperm (nungu), mesocarp pulp (fruit pulp) and tuberous seedlings (tuber)⁴. According to literature the fresh pulp is rich in vitamins A and C and the fresh sap is a good source of B- complex vitamins⁴. Palmyrah is a good source of carbohydrate, calcium, magnesium, iron, and fiber but, limited in fat and protein¹. Further research is needed to exploit its full potential that may influence its extensive consumption. The Present investigation was carried out to determine the presence of vitamins, amino acids and minerals in palmyra palm fruit pulp.

MATERIALS AND METHODS

The collected fruits were sorted out in order to remove damaged ones and were separated on the basis of the state of ripeness, similarity in shape and size. The fruits were then washed, weighted, peeled and pulped. The pulp was stored at 4°C and some of the pulp was dried at 60°C for 24 to 48 hrs. The dried pulp was finally milled using pulverizer to pass through 250 µm sieve. The samples were then packaged in polyethylene bag and kept in a refrigerator (4°C) for further use⁶.

Fat Soluble Vitamins - A, D3, E and K Standard Preparation

Standards of Fat soluble vitamins - A, D, E and K were purchased. 10 mg of the standard was dissolved in 1 ml of methanol.

Sample Preparation

One gram of dry powder of palmyra palm fruit pulp was taken. 5 ml of Hexane was added and mixed thoroughly for 10 minutes. The upper hexane layer was transferred to small test tubes and evaporated to dryness. The residue obtained was dissolved in 50 µl of 95% methanol (Mobile Phase: A-Water; B- Methanol).

Water soluble Vitamins – B group Standard Preparation

Niacin, Pyridoxine, Thiamine, and Riboflavin were purchased. 10 mg of the standard was dissolved in 10 ml of methanol. From this, 100 µl was taken and made up to 10 ml with methanol. This was injected into the HPLC.

Sample Preparation

500 mg of dry powder of palmyra fruit pulp was taken and 10 ml of mobile phase A (1 gm of KH₂PO₄ and 1 gm of Acetonitrile made up to 1000 ml with distilled water) was added and kept in sonicator for 30 min at 40°C then made up to 100 ml. 1gm of KH₂PO₄ and 0.5 gm of Acetonitrile made up to 1000 ml with Methanol and used as Mobile phase B.

Analysis of Fat soluble vitamins

HPLC conditions

Shimadzu CLASS-VP V6.13 SP2 instrument was used to carry out HPLC analysis of fat soluble vitamins employing the following conditions: Column 4.6 x 75 mm Phenomenex -C18, 3.5 µm; Mobile phase A=water, B=methanol, Gradient at 0 min 90% B, at 15 min 100% B, at 20 min 100% B, Column wash at 21 min 90% B; Flow rate-1.0 ml/min; UV detector-variable wave length detector 210 nm, standard cell, Column compartment temperature 20°C; Stop time 21 min, Post time 5 min; Injection volume – 5 µl for fat soluble vitamins. Detector was connected to the computer for data processing ⁷.

Analysis of water soluble vitamins

HPLC conditions

A Shimadzu CLASS-VP V6.13 SP2 Area% Report, Version 5.22 High performance liquid chromatography having UV/visible detector was used to carry out HPLC analysis. Separation was performed on Luna C18 Colum. Detector was connected to the computer for data processing. The working condition of HPLC was binary gradient, mobile phase Solvent A (20%) – 0.05 M KH₂PO₄ in water (pH 2.5) and solvent B (80%) – Acetonitrile; flow rate was 0.70 mL/min, injection volume was 5 µl ; the wavelength of the UV/visible detector 285 nm, Column compartment temperature 40°C for water soluble vitamins ⁸.

Determination of Vitamin C and minerals

Ascorbic acid content of palmyra palm fruit pulp was determined by 2, 6-dichlorophenol indo phenol (DCPIP) titration method ⁹. All the mineral solutions were prepared according to AOAC method (AOAC, 2005) and estimated by flame atomic absorption spectrometric method.

Amino acid profile analysis

Amino acids were determined by high performance liquid chromatography (HPLC) according to the OJEC standard method ¹⁰. Pulp sample (10 mL) was hydrolyzed with HCl (6M) in an ampoule containing 10 mg phenol (for protection of tyrosine) at 110 °C for 24 h. After acid hydrolysis, 30 mL of citrate buffer (pH 2.2) were added, and the pH was adjusted to between 0.5 and 1 with a 7.5 M NaOH and pH 2.2 with a 1 M NaOH. The sample obtained was diluted to 100 mL with citrate buffer after adding 1 mL of a norleucine solution 50 µM (as an internal standard). The sample was filtered through a 0.2 µM

nylon filter before being analyzed by HPLC. Sulphur containing amino acids, cysteine and methionine were determined after pre-hydrolysis oxidation with performic acids.

RESULTS AND DISCUSSION

Table 1 and Figure 1 show the result of HPLC analysis of fat soluble vitamins. The presence of 3 peaks corresponds to the vitamins D3, E and K. Of these vitamin E (2 µg), occurred in higher concentration followed by vitamin K (0.4 µg) and vitamin D3 (0.970 µg). Vitamin A (retinol acetate) was found below detectable level.

HPLC chromatogram of the water soluble vitamins in palmyra fruit pulp (Figure 2) showed four peaks corresponding to the four vitamins identified. Their retention time, peak area, height and concentrations are given in table 2. The B complex vitamins detected were niacin, pyridoxine, thiamine and riboflavin. Among these concentration of thiamine was 0.523 µg whereas riboflavin, pyridoxine and niacin were 9 µg, 3.9 µg and 0.5 µg respectively. Vitamin C content was 16.9 mg/100g.

Vitamins are of water soluble vitamins that play important roles in cell metabolism ¹¹. They are eliminated in urine on daily basis and body need a continuous supply of them in diets ¹². Vitamin C is a potent antioxidant and involves in the synthesis of collagen in connective tissues ¹³. Vitamin E is a powerful antioxidant which helps to prevent the damage of cells from free radicals and it is vital to the formation and normal function of red blood cell and muscles ¹⁴.

The various levels of these minerals could be as a result of variation in some climatic factors and may also be due to the different rates at which the elements are taken up from the soil by the plants. Determination of minerals present in palmyra fruit pulp is expressed in Table 3. Among the mineral elements analyzed, potassium (21.5 mg/100g) showed higher concentration, followed by sodium (20 mg/100g), Phosphorus (15 mg/100g), magnesium 10.2 mg/100g), copper (10 mg/100g) , calcium(8.76 mg/100g), zinc (3 mg/100g), and at a lower concentration, iron (1.5 mg/100g). The elements found in palmyra pulp have great importance for human consumption. The study revealed that palmyra palm fruit pulp contain 9 amino acids (Table 4), including 3 EAA such as lysine (0.8g), phenylalanine (0.41 g) and tryptophan (3.3 g) and 6 NEAA namely aspartic acid (1.6 g), glutamic acid (0.4g), alanine (0.7g), proline (6.3 g), asparagine (2.6 g) and glutamine (0.41 g).

Table 1: Content of fat soluble vitamins of palmyra palm fruit pulp sample

UV Detector (210nm)					
Pk#	Retention time	Area	Height	Concentration µg/100g of sample	Name
1.				0.000 BDL	Vitamin A (Retinol Acetate)
2.	13.750	178347	11414	0.970	Cholecalciferol (D3)
3.	20.367	99241	2098	2	Alpha-Tocopherol (E)
4.	22.858	3743	5	0.4	Vitamin K

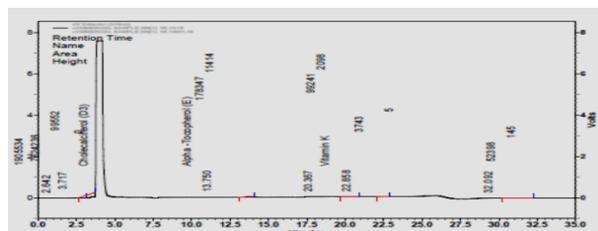


Figure 1: HPLC chromatogram of fat soluble vitamins in palmyra fruit pulp sample

Table 2: Content of water soluble vitamins of palmyra palm fruit pulp sample

UV Detector (285nm)					
Pk#	Retention time	Area	Height	Concentration $\mu\text{g}/100\text{g}$	Name
1.	8.717	132	0	0.5	Niacin
2.	12.567	27059	59	3.9	Pyridoxine
3.	22.667	50558	0	0.523	Thiamine
4.	26.092	5086	0	9	Riboflavin

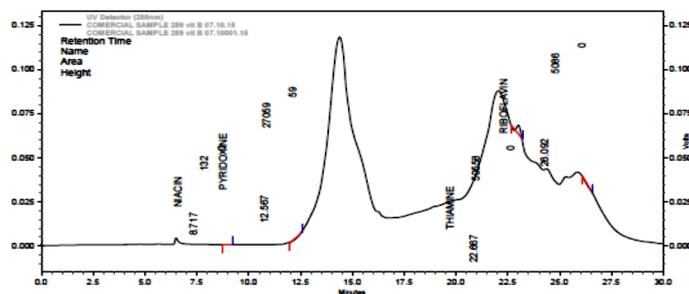


Figure 2: HPLC chromatogram of water soluble vitamins in palmyra fruit pulp sample

Table 3: Mineral composition of the palmyra fruit pulp

Elements	concentration mg/100g
calcium	8.76
phosphorus	15
Iron	1.2
Magnesium	10.2
Zinc	3
Copper	10
sodium	20
Potassium	21.5

Table 4: Amino acid profile of palmyra palm fruit pulp

Amino Acid	Amino Acid (g/100g protein)
Aspartic Acid	2.6g
Glutamic Acid	0.41g
Alanine	0.7g
Proline	6.3g
Phenylalanine	0.41g
Lysine	0.8g
Tryptophan	3.3g
Asparagine	2.6g
Glutamine	0.41g

CONCLUSION

According to the results it can be concluded that the palmyra palm fruit pulp contain good amount of ascorbic acid, minerals and EAAs. The study further revealed compared to various fruits palmyra palm is the cheap, highest source for vitamin C, minerals and EAAs.

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