Studies on *Achras Sapota.* Part 1. The amino acids and carbohydrates contents of the fruits of *Achras Sapota*

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Summary: The amino acids and carbohydrates present in the fruits of Achras sapota have been extracted and identified. The 95, 85 and 70% ethanol extracts afforded the mono- and dissacharides alongwith amino acids, while stepwise extraction with water at room temperature, hot water. ammonium oxalate and sodium hydroxide solutions gave the polysaccharides. The mono- and disaccharides were identified as glucose, fructose, sucrose, lactose and galacturonic acid. The major constituents of the polysaccharides were galactose, galacturonic acid and arabinose with rhamnose and xylose as minor constituents.

The amino acids found to be present were arginine, aspartic acid, alanine, isoleucine, leucine, glutamic acid, glycine, Methionine, Phenylalanine, proline, hydroxyproline, threonine, taurine, tyrosine, serine, valine, phosphoethanolamine alongwith urea.

Introduction

Chicu, a potoato like fruit in appearance and size belongs to a family known as sapotaceae and is called by the following botanical names: *Achras sapota,* A. zapota and A. zapotilla. It is found to grow in most of the tropical countries e.g. Phillipines, West Indies, Ceylon, Malaya, India, Pakistan and Bangladesh. The fruit of chicu is variable in form, normally round or oval, two to three and a half inches in diameter. The flesh is yellowish brown, tender, granular, soft, sweet and delicious without any trace of acidity when ripe. In the unripe state it contains tannins and milky latex. It has become a popular fruit in recent years and jams, sherbets and syrups have been prepared from it[1]. The furits are said to have positive effects on the biliousness and febrile attacks, while the seeds are known to be diuretic and the bark as a tonic and febrifuge[2], The gum known as chicle is used in the preparation of surgical tapes, dental supplies and in the manufacture of chewing gum[3]. The bark contains appreciable amount of tannins (about 11.8%); and is used in coloring ships, sails and fishing appliances[4]. Recently it has been reported that the aqueous extract of the bark is tuberculosis static[5]. Keeping in view its industrial and medicinal uses we were prompted to investigate the chemical constituents of Achras sapota plant and report below the results of our studies on the amino acids and carbohydrate contents of the fruits.

Experimental

Pieces of fresh fruits (1kg) were subjected to extraction by soaking them for 96 hours in 2.5 litres each of 95, 85 and 70% ethyl alcohol succesively to extract soluble sugars and amino acids. The three alcoholic extracts were combined and concentrated under vacuuo to about 150ml (205g) at 40 to 45°C. The viscous extract so obtained was studied for the contents of amino acids & sugars.

Separation of Sugars from Amino Acids: A sample of concentrated alcoholic extract (3.0g) obtained above was subjected to ion exchange chromatography using freshly regenerated Amberlite IR - 120 resins to separate sugars from amino acids. The sugars were eluted with deionised water and the eluates were tested by Molisch test till all the carbohydrate contents were removed. Ninety fractions of about 25ml each were collected at the rate of 250ml per hour. These elutes were also tested for amino acids and were found to give negative results. All the sugar containing fractions were combined and concentrated under vacuuo at 40° to about 5 ml and the concentrated mass, labelled as FC-1, was kept in

deep freezer for further studies.

The amino acids were eluted successively by 5 and 10% ammonia. Over sixty fractions of 25ml each were collected at a flow rate of 125ml per hour, out of which 43 fractions gave purple colour with ninhydrin after removal of ammonia. These fractions gave negative tests for carbohydrate. The sequence of elution of amino acids was determined by measuring the absorbance of each fraction at 375nm. Fractions (94 to 130) containing most of the amino acids were combined and concentrated under vacuum between 40 - 45° and volume made up to 5ml. The concentrate labelled as FA-1 was kept under refrigeration until use.

Qualitative Analysis of Amino Acids: The qualitative analysis of the amino acids in the concentrate FA-1 was achieved by paper chromatography using single and two dimension techniques. The solvent systems employed were n-butanol:acetic acid:water (4:1:5; upper layer) in the first and single dimension; and phenol:water (8:2) in the second dimension respectively. Freshly prepared Cd-ninhydrin solution was used as spray reagent. The amino acids present in the sample were identified as alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine.

Quantitative Analysis of Amino Acids: The quantitative analysis of amino acids was achieved using Beckman's 120-C Automatic Amino Acid Analyser. The amino acids present alongwith their quantities are shown in Table I.

Qualitiative Analysis of Sugars: The qualitative analysis of sugars present in the fruits of Achras sapota was accomplished by means of paper chromatography using dscending technique. Whatman Filter Paper No. 1 (46x57 cm sheets) was spotted with samples of the concentrate FC-1 and reference sugars using following solvent system :

A. n-Butanol:acetic acid:water (4:1:5; upper layer — run for 20 hrs.
B. n-Butanol:ethanol:water (4:1:5; upper layer) — " " 48 hrs.
C. Ethyl acetate:pyridine:water (10:4:3) — " " 11 hrs.
D. Isopropanol:butanol:water (7:1:2) — " " 20 hrs.

Table I. Quantitative Analysis of Amino Acids Present in Fruits of *Achras Sapota.*

No.	Amino Acid	Quantity in moles/100g.
1	Alanine	2.8
2.	Arginine	16.9
3.	Aspartic Acid	4.6
4.	Glutamic Acid	3.7
5.	Glycine	13.8
6.	Hydroxyproline	: :
7.	Isoleucine	2.2
8.	Leucine	2.5
9.	Lysine	10.5
10.	Methionine	0.8
11.	Phenylalanine	trace
12.	Proline	41.2
13.	Serine	10.4
14.	Taurine	: :
15.	Threonine	1.6
16.	Tyrosine	0.8
17.	Urea	: :
18.	Valine	3.5

: : Indicates that the amino acid was present but the quantity could not be determined since standard was not available.

The chromatograms were air dried and sprayed with a modified aniline phthalate reagent developed in this laboratory (phthalic anhydride - 1 gm and 1 ml. of aniline were mixed together and dissolved in 50ml of ethanol:methanol, 3:2 mixture by refluxing on water bath for 10 minutes). The chromatograms were allowed to air dry again after spraying and then kept in oven at 120°C for about 15 minutes. Of the six sugars found to be present in the sample, five were identified as galacturonic acid, glucose, fructose, sucrose and lactose.

Quantitative Analysis of Sugars: For the quantitative analysis of the sugars isolated and identified above, the Smith's phenol - sulphuric acid method[6] based on spectrophotometry was used. 50 µl of the concentrated sample FC-1 was loaded as a 6cms. band on Whatman

Paper No. 3. On either side of this band 5 μl of the sample was also spotted to serve as a marker. The chromatogram was run in the solvent system D mentioned above. The chromatogram was air dried and the two strips from the side containing 5 μl of the sample were cut off from the rest of the paper. The strips were developed with aniline phthalate solution and placed on either side of the chromatogram to locate the position of the various sugars present in the chromatogram. The sugars in form of bands thus located, were cut into small pieces and extracted out by shaking in 5ml of water for four hours. 0.5 ml of each aq. extract was taken in a test tube and to it was added 0.5 ml of 5% phenol and 2.0 ml of conc. sulphuric acid. The contents of the tubes were allowed to cool to room temperature and absorbtion of the pink colouration tht developed in each of the tube was measured at 365nm to determine the concentration of each sugar in the sample. These were found to be as follows: (g sugar per 100g of fruit).

Glucose	3.39%;	Sucrose	2.96%;
		Galacturonic acid	0.48%
Fructose	3.19%;	Lactose	0.48%;
		unidentified sugar	0.05%

Polysaccharides: The polysaccharides present in the fruits of *Achras sapota* were extracted with mild solvents like water, ammonium oxalate and dilute sodium hydroxide in order to isolate the polysaccharides in the original undergraded form. The residue left after extracting the fruits with 95, 80 and 70% ethanol was dried with acetone and treated successively in the following manner:

Extraction with water at room temperature: The residue was treated thrice with distilled water (2.5 litres) at room temperature overnight. The mixture was stirred for homogeneous mixing, centrifuged and decanted. Cold water soluble polysaccharides were precipitated with 50% ethanol, centrifuged and dried by solvent exchange method.

Extraction with hot water: The residual material left after water treatment at room temperature was taken in hot distilled water and stirred at 90° for 3 hrs. The mixture was allowed to cool and then centrifuged. The hot water soluble polysaccharides so obtained were precipitated with 50% ethanol, centrifuged and dried as above.

Extraction with ammonium oxalate: The residue left above was treated thrice with 1% ammonium oxalate (1.5 litre) at 90° for three hrs. each time. The suspension was stirred for homogeneous mixing, centrifuged and decanted. The ammonium oxalate soluble polysaccharides so obtained were precipitated by 50% ethanol, centrifuged and dried.

Extraction with sodium hydroxide: The residual material was finally extracted at room temperature with 20% sodium hydroxide (3 x 1.5 litre) with continuous stirring and centrifuged. The extracted polysaccharides were precipitated with 50% ethanol, centrifuged and dried by solvent exchange method.

Analysis of extracted polysaccharides. Specific rotations of the 1N-NaOH solution of the polysaecharides extracted above were measured at room temperature. Uronic acid anhydride (uaa) contents of polysaccharides were determined by Anderson decarboxylation method[7]. The results of these experiments are given in Table II.

Samples of polysaccharides obtained by different extractions were also examined for the constituent sugars. Samples (20 mg each) were hydrolysed in 1N-sulphuric acid (3 ml) in sealed tubes at 120° for 18 hours. The hydrolysates were then neutralised with barium hydroxide and carbonate. Insoluble were removed by centrifugation. The supernatent was deionised with Amberlite IR-120 (H^+), concentrated and examined by paper chromatography using the solvent systems A,B and C mentioned earlier. The constituent sugars of the various polysaccharides so obtained are shown in Table II.

Results and Discussion

Separation of amino acids and carbohydrates was achieved in the present investigation by action exchange resins. After the separation of carbohydrates from amino acids, their qualitative and quantitative analysis were determined. The amino acids identified by paper chromatography were arginine, aspartic acid, glutamic acid, isoleucine, leucine, lysine, phenylalanine, alanine, proline, serine, tyrosine, threonine, valine and glycine. The quantity of each individual amino acid in the extract was realised through Beckman's Automatic

Table II. Analysis of Various Polysaccharides Extracted from *Achras Sapota.* Fruits (Wt. of the fruits taken: 865Kg)

Sample of polysaccharide extracted from	Weight of the polysaccharide - obtained (g)	%	(uaa) %	$[\alpha]^{rt}_{D}$	Constituent Sugars
Water at room temperature	8.8	1.02	22.0	+240	Arabinose +++ Galactose ++ Gatacturonic acid+ Rhamnose + Xylose (tr)
Hot water	2.3	0.27	19.36	+252	Arabinose +++ Galactose ++ Galacturonic acid + Rhamnose (tr) Xylose (tr)
Ammonium oxalate	10.5	1.21	21.12	+236	Arabinose +++ Galactose ++ Galacturonic acid + Rhamnose (tr) Xylose (tr)
Sodium hydroxide	15.6	1.80	18.48	+260	Arabinose +++ Galactose ++ Galacturonic acid + Rhamnose (tr) Xylose (tr)

+++ = very intense spots on chromatogram.
++ = intense.
+ = low intensity.
(tr) = trace or dim spots.

Amino Acid Analyser which also revealed the presence of additional amino acids that could not be resolved by paper chromatographic methods (see Table I). It is worth noting that Xavier and co-workers[8] had earlier reported the predominance of arginine while our studies indicates that proline is found in maximum quantity in the fruits of *Achras sapota.* followed by arginine. Xavier's results were based solely on paper chromatography and it is probable that they could not have accurately estimated the proline due to two factors i.e. firstly proline and hydroxyproline in which the amino group forms pyrolidine ring and gives condensation products with ninhydrin of different type and colour, and secondly the color so produced is dim and fades very fast. In case of Amino Acid Analyser, the amino-acids ninhydrin adduct do not come in contact with air and thus remain stable for much longer period enabling accurate spectrophotometric determinations. It is also possible that the difference

between our results and that of Xavier's is due to the effects of different climatic and soil conditions. The results in the table also indicates that amino acids and proteins occur in substantial amount in the fruits of Achras sapota thus giving it a high nutritive value.

Amongst the free sugars found to be present in A. sapota fruits are fructose, glucose, lactose, sucrose and galacturonic acid. A sixth sugar was also found to be present but its identity could not be established in the present studies. Glucose and fructose had also been identified to be present by previous investigators[9]. Evidence of a sugar resembling lactose too has been reported[10].

No detail studies have until now been reported on the polysaccharide contents of A. sapota fruits and the present investigation is probably the first attempt in this direction. The polysaccharide components of fruits were extracted stepwise with water at room temperature, hot water, 20% sodium hydroxide and ammonium oxalate according to the method described by M. Uddin[11] and us[12]. Acid hydrolysis of samples of polysaccharides so extracted, followed by paper chromatography examination, revealed that the principal constituent sugars of all the polysaccharides were same and were found to be arabinose, galactose and galacturonic acid alongwith rhamnose and xylose as minor components. Specific rotations and uaa percentage of each sample (see table II) further indicated that all the polysaccharides of *Achras sapota* are probably of polydisperse type comprising of a family of polymers having more or less the same structural pattern.

Beside the above findings we have also found several n-hexane soluble substances. Preliminary investigations reveal them to be sterols and triterpenoids. We are presently engaged in isolating and purifying these constituents and hope to report our findings later.

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