The Free Amino Acids of Fresh Juices of Pakistani Citrus Fruits

Manzoor Elahi and Nawab Khan

Compositional studies with respect to sugars, acidity, pH, Brix value, and formol value were made on fresh juices of six citrus fruits. These were also analyzed for free amino acids by paper chromatography and in all 15 amino acids were identified with different solvent systems. Free amino acids were aslo estimated quantitatively. Glycine was found only in Mosambi oranges. Free amino acids of Valencia Late oranges were compared with those of California Valencia and Israel Shamouti oranges.

Browning of citrus juices during processing is one of the problems which plagues the processor because it adversely affects the acceptability of the products due to the development of off-flavors and objectionable colors. This browning is primarily the result of the reaction between amino acids and reducing sugars, and is dependent on various factors like pH, temperature, and concentration of the reactants. Therefore, a study of the reducing sugars and amino acids of citrus juices becomes imperative and would help the industry in estimating the maturity of initial fruits and the quality of the products.

The free amino acid content of American (Rockland, 1959, 1961), Italian (Wucherpfenning and Franke, 1966; Safina, 1953), Israeli (Coussin and Zdenka, 1968), and Japanese (Ito and Sakasegawa, 1962) orange varieties has been reported in literature. It has been used as an index of maturity (Rockland and Underwood, 1954; Wedding and Horsepool, 1955) and quality (Safina, 1964) for citrus fruits and juices, respectively. Some reference to their possible nutritional (Rockland, 1961) importance has also been made.

No information is yet available on the free amino acids and reducing sugars content of Pakistani citrus fruits. The present paper reports the qualitative and quantitative estimation of free amino acids by paper chromatography. It also contains the estimation of total reducing and nonreducing sugars.

EXPERIMENTAL

Apparatus and Reagents. Paper chromatography was carried out on Whatman No. 1 paper using a glass chromatography chamber (21 × 8 in.). pH was measured by a pH meter (Model 23A, Electronic Instruments Ltd., Richmond Surrey). Brix measurements were made with a sugar refractrometer (Bellingham and Stanley Ltd., London). Absorbance readings were taken with Beckman DU spectrophotometer.

Ninhydrin reagent was prepared by dissolving the commercial product without purification as a 0.5% solution in distilled acetone. Solvents used were of analytical grade.

P.C.S.I.R. Laboratories, Ferozepur Road, Lahore-16, West Pakistan.

PROCEDURE

Preparation of Sample. Six varieties of citrus fruits (Mosambi, Valencia Late, Blood-Red, Sangtara, Grapefruit, and Kinnow) were purchased from the market in early March, 1970. They were washed with water and hand-peeled. The juice of each variety was extracted with a juice extractor and passed through a 16-mesh stainless screen to make it fiber-free. The physical and chemical measurements were made on the juice of each variety. Standard methods of AOAC (1960) were employed for the determination of acidity, reducing, and nonreducing sugars. The formol value was estimated by the method of Safina (Safina, 1964). The results have been recorded in Table I.

For chromatographic analysis of the amino acids the juice samples were filtered by suction through a Buchner funnel on Whatman No. 1 paper (Coussin and Zdenka, 1968).

Qualitative Analysis of Free Amino Acids. Amino acids were separated by an ascending one-dimensional paper chromatographic technique. The following solvent systems were used:

Solvent 1. *n*-Propyl alcohol-water (70:30 v/v)
Solvent 2. Phenol-*n*-propyl alcohol-water (5:1:1 v/v)
Solvent 3. Methyl ethyl ketone-propionic acid-water-*tert*-butyl alcohol (75:25:30:25 v/v)

Preliminary experiments were performed to standardize the conditions for the separation of pure amino acids. Out of the amino acids used, the following pairs were not distinctly separated in solvent 1: serine and glycine; valine and methionine; and alanine and threonine.

The first two pairs were separated in solvent 2, while the third one is separable by first passing solvent 1 and then, after drying, running in solvent 3 in the same direction.

The separation of free amino acids consisted of spotting about 1 ml of the filtered juices 5 cm from the bottom of the paper along with pure amino acids, and drying each spot in a stream of warm air. The atmosphere of the developing glass chamber was allowed to equilibrate with the solvent for 12 hr prior to development and fresh solvent was used for each run. The papers were run for 24 hr, removed, and allowed to dry at room temperature. The chromatograms were sprayed with ninhydrin reagent and spots were made visible by heating at 70° C. R_f values were calculated as shown in Table II.

Table I. Composition of Untreated Fresh Juices of Some Citrus Fruits

Name of Fruit	Botanical Names	pH	Acidity as citric acid %	Brix %	Reducing sugars %	Non-reducing sugars %	Total as reducing sugars %	Formol value ml *M*-NaOH/l. Juice %
Valencia Late Orange	Citrus *Aurantium* (Var. Valencia Late)	4.5	0.52	8	3.24	1.77	5.01	14.2
Blood-Red Orange	(Var. Blood-Red)	4.4	0.54	9	4.44	1.54	5.98	13.0
Mosambi Orange	(Var. Mosambi)	4.8	0.24	9	6.16	0.48	6.64	25.0
Sangtara	(Var. Sangtara)	3.4	0.49	10	4.6	4.5	9.1	19.0
Kinnow	(Var. Kinnow)	4.3	0.75	10	3.5	2.58	6.08	23.0
Grapefruit	Citrus *Paradisi*	3.6	1.27	6	3.6	0.59	4.19	18.0

Identification of amino acids was made according to R_f values and ninhydrin color reactions, compared with those of pure compounds treated under similar conditions.

Quantitative Analysis. Chromatographic runs were carried out as previously described by spotting known volume (1 ml) of each filtered juice and the ninhydrin color spots were cut out from the papers and extracted in test tubes in a known volume (10 ml) of 95% ethyl alcohol. Absorbance readings were recorded at 570 mμ against a blank eluted from one of the papers. Quantitative estimations were then made by comparing the results with standard curves previously obtained on pure amino acids under the same conditions. The results have been recorded in Table III.

Table II. The Free Amino Acids Identified in Fresh Juices of Different Citrus Fruits-R100F Value

Amino Acid	Solvent Systems 1	2	3	1 and 3
Leucine	0.74	0.75	0.55	
Phenyl alanine	0.71	0.71	0.51	
Valine	0.62	0.65	0.43	0.84
Methionine	0.61	0.64	0.41	0.62
Proline	0.58	0.47	0.42	
Tyrosine	0.55	0.52	0.38	
Alanine	0.43	0.43	0.23	
Threonine	0.42	0.32	0.21	
Glycine	0.32	0.30	0.17	
Serine	0.31	0.20	0.15	
Arginine	0.24	0.46	0.14	
Lysine	0.21	0.39	0.10	
Asparagine	0.18	0.02	0.14	
Glutamic Acid	0.15	0.10	0.21	
Aspartic Acid	0.12	0.06	0.15	

DISCUSSION

Table I shows the composition of some fresh citrus juices. It is interesting to note that with its lowest acidity among the citrus *Aurantium* varieties, Mosambi contains the maximum percentage of reducing sugars and the minimum of nonreducing sugars. Formol value varies from 13 to 25, with Mosambi having the maximum.

The free amino acids identified in the fresh juices of citrus fruits are listed in Table II along with their R_f values. In all, 15 amino acids have been identified. Proline, phenyl alanine, aspartic acid, tyrosine, valine, serine, and DL-alanine have been found in all the juices. Valencia Late variety contains the maximum (*i.e.*, 12) and Kinnow the minimum number (*i.e.*, 9) of free amino acids. It is interesting to

Table III. Quantitative Estimation of Free Amino Acids in Untreated Fresh Juices of Different Citrus Fruits

Name of Fruit	Botanical Names	Amino Acids (mg/100 ml juice) Proline	Aspartic acid	Tyrosine	Valine	Serine	Alanine	Phenyl alanine	Threonine	Glycine	Leucine	Methionine	Lysine	Glutamic acid	Asparagine	Arginine
Valencia Late Orange	Citrus *Aurantium* (Var. Valencia Late)	8	55	12	10	12	14	12	20	...	...	...	2	25	12	28
Blood-Red Orange	(Var. Blood-Red)	10	50	16	20	32	10	12	28	...	...	...	...	32	56	36
Mosambi Orange	(Var. Mosambi)	12	60	32	15	16	30	14	...	115	33	...	20	...	...	48
Sangtara Orange	(Var. Sangtara)	10	58	19	24	24	16	7	...	...	4	...	...	...	16	38
Kinnow Orange	(Var. Kinnow)	15	13	16	21	14	3	...	12	...	...	...	6	...	...	14
Grapefruit	Citrus *Paradisi*	6	58	18	30	22	14	6	...	...	3	12	8	...	...	...

note that glycine has been identified in considerable amounts only in Mosambi variety.

Table III records the concentration of free amino acids as estimated by colorimetric technique. Aspartic acid is found in substantial amounts in all the fruits. A comparison of the content of eight free amino acids (*i.e.*, alanine, arginine, aspartic acid, glutamic acid, leucine, lysine, serine, and valine) of Valencia Late variety with those of California Valencia shows that the former contains them in less amounts. It will also be observed that aspartic acid is the most abundant acid in Valencia Late, while California Valencia (Rockland, 1959) contains arginine in maximum amounts. These discrepancies might be due to varietal differences or to cultural and environmental factors. The processing and storage of fruit juice before chromatographic separation may, in addition to the above mentioned factors, be responsible for the higher free amino acid content of Israel Shamouti orange (Coussin and Zdenka, 1968) than that of Valencia Late variety.

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