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STUDIES ON AMINO ACIDS OF *CARISSA CARANDAS*

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The amino acid composition of the seeds and pulp from the furits of *Carissa carandas* was studied using automatic amino acid analyzer which significantly revealed the presence of only six free amino acid in pulp juice. The amino acid pattern is highly specific and may be used as an authentic index for evaluation of adulteration. The analysis of ash of the fruit indicated large quantity of potassium ions.

Key words. *Carissa Carandas,* Amino acids, Composition.

Introduction

Carissa carandas, a wildly grown shrub in the subcontinent produces berry like white to red and maroon shaded fruits commonly known as Karonda. The fruits are eaten raw when partially or fully ripe. The fruits are pickled in vinegar or salt and sometimes are syruped in sugar solution for preservation.

The fruits have never been studied for their nutritional or food values, although their pharmacological characteristics are well explored [1]. The various organs of the plant such as root, bark, leaf and fruit have been found effective medicinally [2-5]. Some of the components have also been isolated from the fruits as volatile oils [6], organic acids [7,8] and sugars [9]. The leaf extract has exhibited certain enzymic activities close to diastase, invertase and protease [10].

Plants usually contains, in varying proportions the full range of mineral elements which are present in the soil in which they are grown. The mineral elements play an influential and pivotal role in determining the quality of fruits and vegetable products. Apart from the impacts of deficiencies of essential elements during growth, the levels of particular minerals can also be affective in the post harvest behavior of constituents in pome fruits, which are often stored for long periods of time before reaching to the retail market for consumption, calcium is mainly associated with the pectic materials of the cell-sap and its association with pectic polymers in maintaining the rigid texture of cell wall in fruits and vegetable is now well understood.

The present study deals mainly with the protein and amino acids of the fruit juice and seed extracts. The free (unhydrolyzed) and the bound amino acids (hydrolyzed) have been determined by using automatic amino acid analyzer. Protein contents in seeds and fruit pulp were estimated and compared with other fruits.

Material and Methods

Analytical grade chemicals and double distilled water were used through the study. Fresh fruits were obtained from the trees on the sideways in the university campus and juice was extracted by hand pressing. Protein content was determined by micro-Kjeldahl apparatus and minerals were estimated by a flame analyzer (Gallenkamp).

Amino acid analysis. Free amino acids (FAA) were analyzed in the juice by taking 2 ml of the juice mixed with 2 ml of 10% sulfosalicylic acid. It was shaken vigorously and was kept for 30 min. at 4° in a refrigerator to precipitate the proteins or peptides present and finally centrifuged at 3000g. The clear supernatent (25 µl) was used for the analysis using biotronic automatic amino acid analyzer (LC-6001, W. Germany).

A known quantity of pulp and seeds was ground in water separately, stirred continuously for 2 hr and filtered. The filtrate was concentrated to one third and equal quantity of ethanol was added for precipitation of proteins. The precipitated proteins were centrifuged and dried.

Amino acid composition of protein fractions were determined by taking one gram of sample in 2 ml of 6N HCl in a tube which was sealed under vacuum. The sealed tube was incubated at 110° for 20 hr to ensure complete hydrolysis of the proteins. The excess reagent was removed by distillation under reduced pressure and dried mass was dissolved in 1 ml of sodium citrate buffer. The amino acid compsotion of each sample was determined by taking 25 µl for each test.

The minerals sodium, potassium and calcium in ash were estimated by flame analyzer (Gallenkamp model FGA-330). Fresh fruits (5 g) were weighed in a silica dish which was previously ignited and cooled before weighing. Then the dish and contents were ignited gently over a low flame until charred and then in a muffle furnace at 550° overnight. The ash content of karonda fruit was found to be 1.13 %.

Results and Discussion

The fruits of *Carissa carandas* contain about 65% juice having pH 2.9 and a brix value of 7.5 (Table 1). The high acidity of juice is responsible for astringency in taste.

TABLE 1. ANALYSIS OF KARONDA JUICE.

Juice	64 ml/100g fruit
pH	2.9
Brix	7.5
Protein (Pulp)	0.8 %
Protein (Seed)	3.5 %
Ash	1.13 %

TABLE 2. AMINO ACID COMPOSITION OF *CARISSA CARANDAS*.

Amino acids	Pulp (m.mole/100g)		Seeds (m.mole/100g)	
	F.A.A.	Hydrolyzate	F.A.A.	Hydrolyzate
Serine	4.71	8.93	379.68	95.95
Proline	7.93	-	34.42	-
α-Amino butyric acid	1.42	-	28.78	-
Methionine	3.32	-	10.7	-
γ-Amino butyric acid	0.31	1.73	111.88	-
Histidine	5.75	-	19.16	10.87
Valine	-	13.03	-	-
I. Leucine	-	5.69	7.3	-
Leucine	-	10.26	6.08	-
Tyrosine	-	3.39	3.46	-
Phenylalanine	-	5.96	-	-
Glutamic acid	-	6.79	-	80.12
Threonine	-	10.59	-	-
Glycine	-	28.89	-	61.34
Alanine	-	35.33	-	34.91
β-Alanine	-	-	33.92	-
Lysine	-	-	7.93	22.93
Aspartic acid	-	-	-	62.54

F.A.A. = Free amino acid; -- = Not detected.

However the juice has higher shelf life because of significantly low pH. The pulp and seeds contain 0.8 and 3.5 % protein respectively. A significant amount of minerals including sodium 4, potassium 360 and calcium 38 mg/100g fresh material were found in seeds. The extra large quantity of potassium reflects the nutritive value of fruits.

The amino acid profile shows the presence of only six free amino acids in pulp out of which the two amino butyric acids are non-proteineous. Moreover the free amino acid in pulp are only in minute quantity. Serine is the only amino acid present in pulp and seed in hydrolyzed as well as unhydrolyzed form, although it is abundantly present in seeds as free amino acid. The proline does not take part in protein biosynthesis and is only present in unhydrolyzed extract of pulp and seeds. Valine, phenylalanine, threonine and β-alanine are exclusively present in pulp while aspartic acid and lysine appear in seeds only. The large quantities of serine and α-amino butyric acid make the amino acid profile specific for fruits of *Carrisa carandas*. Aspartic or glutamic acid including their amides asparagine and glutamine appear to be especially abundant in many species of citrus fruits including tomato. The berry fruits as strawberry, gooseberry and blackberry are also rich in these amino acid while potato may also be quoted for having significant amount. Asparagine is also by far the most abundant individual constituent of the non-protein nitrogen fraction. Pears and oranges are especially rich in proline, while black and red currants contain alanine in excess.

All the common amino acids of proteins are however, present in the fruit although found only in trace amounts. It has been recently discovered that two of non protein amino acids β-alanine and γ-amino butyric acid are very widely distributed in some fruit and vegetable tissues [11].

Potassium is the most abundantly found mineral element in fruit and vegetables, it is usually between 60 and 600 mg/100 g of fresh material representing over 1% of the fresh weight in some products as parsley [12]. The potassium contents of karonda as 360 mg/100 g show significant nutritive importance of the fruits (Table 3). The fruits show high potentials for commerciallization in fruit juice and other food industries.

TABLE 3. MINERAL CONTENTS OF KARONDA FRUITS.

Element	In Karonda fruits (mg/1000 g of fresh material)	Aproximate normal range of concentration(mg/100g fresh material)
Sodium	4	0 - 124
Potassium	360	60 - 600
Calcium	38	3 - 300

References

1. M. L. Chatterjee and A. R. Roy, Bull. Calcutta School Trop. Med., **13** (1), 14 (1965).
2. S. N. Joglekar and B. B. Gaitonde, J. Jap. Pharmocol., **20** (3), 367 (1970).
3. A. Y. Zaki, S. F. El-Tohamy, M. Hashem, and Abd El-Fattah, J. Egyp. Pharm. Sci., **22** (1-4), 113 (1981).
4. W. Dymock, C. J. H. Warden and D. Hopper, *Pharmacographia Indica* (Kegan Paul, Trench trubno and Co. Ltd., London, 1980), vol. 2nd, pp. 419.
5. G. Watt., *A Dictionary of Economic Production of India* (Cosmo Publication, Delhi, India, 1972), vol. 165.
6. A. Y. Zaki, S. F. El-Tohamy and S. A. El-Fattah, Egypt. J. Pharm. Sci., **22** (1-4), 127 (1981).
7. S. Kumar and M. P. Tandon, Indian Phytophathol., **32**, (1), 134 (1979).
8. R. C. Rastogi, R. P. Rastogi and M. L. Dhar, Ind. J. Chem., **5** (5), 125 (1967).

9. M. Daniel and S. D. Sabnis, Ind. J. Exp. Biol., **16** (4), 512 (1978).
10. A. Lal and R. Pab Singh, J. Ind. Boton. Soc., **32**, 54 (1953).
11. J. Bonner, *Plant Biochemistry* (Academic Press, New York, 1950).
12. R. B. Duckworth, *Fruit and Vegetables* (Pergamon Press, Oxford OX3 OBW, England, 1979).