Free Amino acids profile of ripening Cherimoya fruit

Contenido de aminoácidos libres en frutos de chirimoyo durante la maduración

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RESUMEN

El chirimoyo es un fruto tropical que crece en algunos países de América y en una serie de selectivas áreas del sur de España, siendo una fuente rica de aminoácidos libres. Los efectos de las temperaturas después de la postrecolección de este fruto han sido estudiadas. La firmeza del fruto se ha determinado con un medidor de textura y los aminoácidos libres se determinaron por cromatografía en columna de intercambio iónico, utilizando un autoanalizador de aminoácidos. El chirimoyo se caracterizó por un alto contenido en prolina y citrulina. El ácido glutámico y la glutamina estaban también presentes pero en pequeñas cantidades. La citrulina y la prolina se incrementaron durante la maduración; el aumento dependió de la temperatura de almacenamiento.
Palabras clave: Aminoácidos, Chirimoyo. Fruto tropical

ABSTRACT

Cherimoya, a subtropical fruit growing in some American countries and restricted areas of the south of Spain, is one of the richest sources of free amino acids. The effects of post-harvest temperatures on the texture and free amino acid profile of this fruit were studied. Fruit firmness was determined with a pressure tester, and free amino acids were measured by ion-exchange colum chromatography using an automatic amino acid analyzer. Cherimoya was characterized by high contents of proline and citrulline. Glutamic acid and glutamine were also present but at lower levels. Citrulline and proline increased during ripening; the increases depended on the temperature of storage.
Key words: Amino acids, Cherimoya, Tropical fruits.

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INTRODUCTION

Cherimoya fruits (*Annona cherimolia)* after harvesting at the green-ripe stage are usually ripened at room temperature (20-22 °C) during 2-3 days. Marketing troubles quickly develop, mainly due to softness and brownig of the fruits, although changes in texture depend on conditions of ripening. To extend the shelf life of cherimoya the storage of fruits at 10 °C and 85% relative humidity has been suggested (1).

Annonaceae fruits are one of the richest sources of free amino acids and a great interest in their study has been shown in the last few years. Ventura and Lima (2) have examined sugar apple *(Annona squamosa)* which is unusual and metabolically interesting in that γ-aminobutyric acid and citrulline predominate among a total of 14 amino acids, including arginine and ornithine. In the soursop *(Annona muricata)* proline and γ-aminobutyric acid are the major amino acids, with nine others in smaller amounts. *Annona ch. crassiflora* and *Annona paludosa* contain large amounts of basic amino acids, mainly histidine, lysine, arginine and ornithine. Citrulline and α- ß-diamine butyric acid are present in lower concentrations (3).

The present paper deals with the effects of different post-harvested ripening temperature on the texture of cherimoya fruits as well as on the qualitative and quantitative free amino acid profiles of epicarp and mesocarp.

MATERIALS AND METHODS

Materials

Uniform weight (500 + 50 g) cherimoya fruits of the "Fino de Jete" variety were harvested at the green-ripe stage of maturity and subsequently ripened under the following conditions: 20-22 °C and 85% relative humidity (R.H.); 11 °C and 85% R.H.

The fruits were stored during 12 to 16 days depending on the temperature of ripening. Each four samples were taken to measure texture and to determine contents of free epicarp and mesocarp amino acids.

Texture measurements

Fruits firmness, expressed at Kg/cm^2 pressure, was determined with a GILFA pressure tester Mod. 1 (Granada, Spain). Whole intact fruits were puntured by using a cilindrical flat-surface plunger with a 12 mm. diameter. Three readings were taken per fruit, in the mid region at a 90 °C angle to the longitudinal axis.

Amino acids analysis

The fruits were washed with tap water and dried with filter paper. Mesocarp was separated from the epicarp with a stainless steel spatula and seeds were removed. Each tissue was cut into small pieces, weighed and macerated using a Sorvall-Omnimixer homogenizer during 5 min. Sample homogenization was carried out with approximately 100 g. of mesocarp or 50 g. of epicarp and two volumes of 80% methanol, containing 0.2% sodium disulphite. The suspensions were filtered twice through four layers of surgical gauze, resuspended and disagregated using one volume of 0.2% sodium disulphite in 80% methanol, in a similar way to the first homogenization. The pooled methanolic extracts were centrifuged at 1200 x g. and vaccuum concentrated at 40-50 °C.

Final volumes for epicarp and mesocarp extracts were respectively 100 ml and 50 ml. Samples for amino acids analysis were diluted five times with a free-salt buffer pH 2.1 containing 250 nmoles of Norleucine as internal standard.

Amino acids were determined by ion-exchange column chromatography using a Chromaspeck-J-180 automatic amino acid analyzer as described by Rank-Hilger (4).

STATISTICAL

Differences for mean amino acid concentrations were assesed by a double-way analysis of variance; sources of variations were storage temperature and storage time.

RESULTS

The breakdown pressure decreased at 20-22 °C and 11 °C with the storage time to a limit value and afterwards this value remained approximately constant (Fig. 1). Decreasing of breakdown pressure at 11 °C had a lower slope, than at 20-22 °C. The breakdown pressure limit was reached after 4 days of ripening at 20-22 °C and after 12 days at 11 °C.

The total free amino acids content of both epicarp and mesocarp increased significantly ($p<0.05$) from the green-ripe to the climateric stage at 20 °C. This also was true for epicarp at 11 °C but the total free amino acids content in cherimoya mesocarp remained fairly constant (Table I).

Tables II and III show the free amino acid contents of epicarp and mesocarp for cherimoya fruits ripened at 20 °C and 11 °C and 85% relative humidity after harversting at the green ripe stage.

At harvest citrulline concentrations accounted for 38.2% and 33.2% of the total free amino acids of epicarp and mesocarp, respectively. Proline accounted for 28.6% and 23.4% respectively and glutamic acid was present in 7.4% in mesocarp but its concentration in epicarp did not reach 1%. On the contrary, ornithine accounted for 7.5% of total epicarp free amino acids while in mesocarp accounted for 0.6%. All the other amino acids were present in approximately the same proportions, oscillating between 0.3-2%.

During storage at 20-22 °C and 11 °C citrulline and proline continued to be the predominant amino acids both in epicarp and mesocarp (Fig. 2 and 3). At room temperature, citrulline slightly increased from the harvest to the senescence period in cherimoya mesocarp. This increase was not evident in epicarp (Table II). At 11 °C citrulline slightly decreased with advancing ripening time for mesocarp (Fig. 4).

At 22 °C, proline constantly increased during ripening and beginning of senescence in cherimoya mesocarp but declined after a long storage time (16 d) (Fig. 2). This amino acid also increased in epicarp from the harvesting to the ripening period but oscillated in concentration during the senescence (Fig. 3).

At 11 °C proline slightly increased in mesocarp and remained constant thereafter (Fig. 4).

Fig. 2 and 3 show the levels of glutamic acid and glutamine in ripening mesocarp and epicarp of cherimoya fruits. In mesocarp the concentrations of these amino acids remained relatively constant during ripening and senescence (Fig. 2). However, in epicarp, glutamic acid and glutamine showed a constant increase in these periods.

Glutamic acid and glutamine concentrations were significantly increased in mesocarp of 11 °C ripened fruits (Fig. 4) as compared to those ripened under room temperature conditions (Fig. 2 and 3), but the levels of these amino acids appeared to be constant during all the ripening time.

All other amino acids, mainly ornithine, contributed to the rise in the level of total free amino acids during ripening, both in mesocarp and epicarp (Table II and III).

DISCUSSION

Ripening of cherimoyas was characterized by a decrease in the breakdown pressure until a limit which was reached after 4 days of harvest at 20-22 °C and after 12 days at 11 °C.

Cherimoyas then appeared ripened for 3-4 days with pink-white flesh and good flavor. Increasing storage time the fruits developed off-flavor and simultaneously the epicarp darked, probably as a result of senescence metabolism.

Our data are in agreement with those obtained by Fuster and Prestamo (4), although these authors did not described the changes in texture, taste and flavors after ripening.

The rapid accumulation of amino acids during the ripening period has been attributed to the decreasing demand for these metabolites in the shoot parts (5).

Annona cherimolia shows a quite different free amino acids profile than those reported for other annonaceae. While the cherimoya is characterized by high contents of citrulline and proline, the soursop contains, in addition to proline, high concentrations of -aminobutiric acid. On the other hand the sugar apple contains significant amounts of citrulline and -aminobutiric acid but the proline does not represent a major free amino acid (2).

Other annonaceae growing in wild areas of the Guyanne such as *A. crassiflora* and *A. paludosa* contain high levels of basic amino acids but the concentrations of free citrulline and proline are more limited (3).

The cherimoya is a metabolically interesting fruit since it contains low levels of γ-aminobutyric acid. This amino acid has been shown as one of the most important metabolites in the ripening and senescence of several fruits (6, 7).

The ripening process of cherimoya appears to be closely associated with the metabolism of proline, citrulline and glutamic acid. The contents of glutamic acid and glutamine in the fruits stored at 11 °C are higher than in those ripened at room temperature. This finding may be attributed to the fact that these amino acids are actively metabolized depending on the temperature of ripening. When the fruits are maintained at 20 °C glutamic acid and glutamine entered into the TCA cycle to meet the requirements of increased energy during the ripening period and to furnish carbon skeletons; both amino acids may also be increased at low ripening temperatures as a consequence of a lower rate of conversion to proline and/or citrulline which are also increased in fruits stored at 20 °C compares with those ripened at 11 °C.

The increase in the citrulline levels during the ripening process may provide a mechanism for the utilization of ammonium excess similar to that suggested for arginine in the ripening grapes (8).

The role of proline in ripening fruits is not fully understood. Rao (9) described an increase in the proline levels of ripening grapes correlated with a decrease in arginine and glutamic acid. However, Pool *et al.* (10) reported a rise in the levels of both arginine and proline during long term storage.

The increase of proline concentrations in several fruits are related to the ripening and senescence periods (6, 11, 12). Proline in cherimoya seems to be also related to these periods being the concentrations dependent of the storage temperature. Amino acids are known to be substances taking part in the reactions of oxidized phenols. Thus, proline as a major cherimoya free amino acid may have a role in the browning reactions taking place during ripening of this fruit.

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Table I.—Total free amino acids contents of epicarp and mesocarp fruit during ripening at 20 °C and 11 °C and 85% relative humidity after harvesting at the green-ripe stage.

	EPICARP		MESOCARP	
Storage time	*Storage Temperatures*			
(days)	*20 °C*	*11 °C*	*20 °C*	*11 °C*
0	22.6	17.8	9.2	28.8
4	34.7	21.1	9.0	26.4
8	26.1	19.2	29.2	23.1
12	39.5	20.0	17.8	25.7
16	—	43.4	—	—

Results are expressed in µmoles/g fresh tissue as mean of four fruit samples.
— Not determined.

Table II.—Free amino acids content of epicarp cherimoya fruit during ripening at 20 °C and 11 °C and 85% relative humidity after harvesting at the green-ripe stage.

Storage time (days)	*Storage temperature*							
	20 °C				*11 °C*			
	0	*4*	*8*	*12*	*0*	*4*	*8*	*12*
Amino acid								
Taurine	0.4	1.4	0.4	1.3	—	0.9	0.6	1.1
α-aminoadipic	0.4	0.5	0.7	1.0	0.8	0.8	0.8	1.6
γ-aminobutiric	—	—	—	—	—	0.6	0.6	0.9
Aspartic	0.6	0.3	0.1	0.3	0.4	0.4	0.3	0.4
Threonine	0.2	0.2	0.3	0.5	1.4	1.6	0.3	0.4
Serine	0.5	0.3	0.2	0.5	0.6	0.6	0.7	1.0
Asparagine	0.8	0.4	0.7	0.9	0.2	1.4	1.4	2.0
Glutamic	0.1	0.3	0.3	0.5	1.9	1.6	1.8	1.7
Glutamine	0.4	0.7	0.9	1.4	1.4	1.8	1.5	1.6
Proline	4.9	12.6	4.5	13.4	3.5	7.5	5.5	5.8
OH-Proline	0.2	0.2	0.4	0.6	0.2	0.5	1.4	0.4
Glycine	0.1	0.1	0.2	0.1	0.1	0.3	0.2	0.2
Alanine	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.2
Citrulline	11.8	14.3	15.9	14.2	—	—	—	—
Valine	0.3	0.2	0.6	0.6	—	—	—	—
Isoleucine	—	—	0.5	0.2	—	0.1	0.1	0.1
Leucine	—	—	0.3	0.2	—	—	—	0.1
Tyrosine	0.1	0.2	0.1	0.3	—	—	—	—
Phenylalanine	0.3	0.1	0.3	0.6	4.4	3.7	3.2	2.6
Triptophan	0.1	0.7	0.1	0.2	—	—	—	—
Histidine	—	—	—	—	—	0.1	0.1	0.2
Ornithine	1.2	1.8	1.6	2.2	2.2	1.5	1.8	1.7
Lysine	0.2	0.3	0.3	0.4	—	—	—	—
Arginine	—	—	—	—	—	—	—	—

— Not detected.

Results are expressed in μmoles/g fresh tissue as mean of four fruit samples.

Table III.—Free amino acids content of mesocarp cherimoya fruit during ripening at 20 °C and 11 °C and 85% relative humidity after harvesting at the green-ripe stage.

Storage time (days)	*Storage temperature*							
	20 °C				*11 °C*			
	0	*4*	*8*	*12*	*0*	*4*	*8*	*12*
Amino acid								
Taurine	0.5	0.1	0.5	0.5	0.5	0.5	0.7	0.7
α-aminoadipic	0.2	0.2	0.6	0.5	1.3	0.7	0.5	1.1
γ-aminobutiric	0.3	0.2	0.7	0.5	—	—	—	—
Aspartic	0.1	0.2	0.2	0.1	0.5	0.5	0.3	0.5
Threonine	0.1	0.1	0.2	0.1	0.3	0.3	0.2	0.3
Serine	0.2	0.1	0.2	0.2	0.7	0.5	0.4	0.5
Asparagine	—	—	—	—	0.2	1.4	1.3	1.8
Glutamic	0.1	0.1	0.1	0.1	1.9	1.3	0.7	0.8
Glutamine	0.2	0.1	0.1	0.1	3.7	2.2	2.0	2.9
Proline	1.9	4.9	17.1	7.2	4.9	6.5	5.0	6.2
OH-Proline	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.4
Glycine	0.1	0.1	0.2	0.2	—	—	—	0.1
Alanine	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.3
Citrulline	3.8	1.7	7.3	6.0	12.3	9.5	9.1	8.6
Valine	0.1	0.1	0.2	0.2	—	—	—	—
Isoleucine	—	—	—	—	—	—	—	0.1
Leucine	0.1	0.1	0.1	0.1	—	—	—	0.1
Tyrosine	0.1	0.1	0.1	0.2	—	0.1	0.1	0.2
Phenylalanine	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Triptophan	0.1	0.1	0.1	0.1	—	—	—	—
Histidine	0.1	0.1	0.1	0.1	0.1	0.3	0.5	0.1
Ornithine	0.9	0.4	1.1	1.3	1.7	1.7	1.6	1.6
Lysine	0.2	0.1	0.2	0.2	—	—	—	—
Arginine	—	—	0.1	0.1	—	—	—	—

— Not detected.
Results are expressed in μmoles/g fresh tissue as mean of four fruit samples.

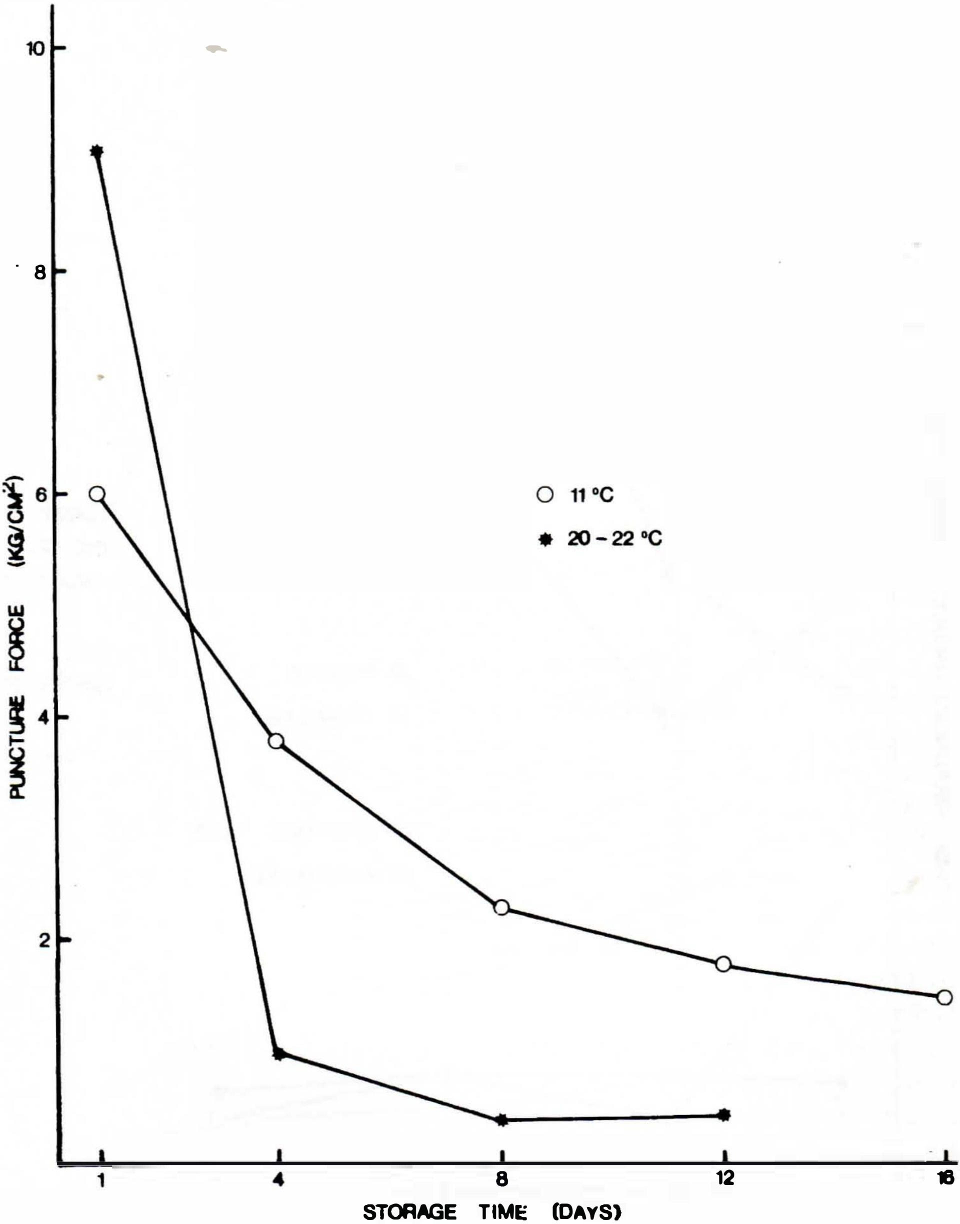

Fig. 1.—Puncture pressure required to breakdown the external skin of cherimoya fruit during ripening at 11 °C and 20-22 °C and 85% relative humidity, after harvesting at the green-ripe stage.

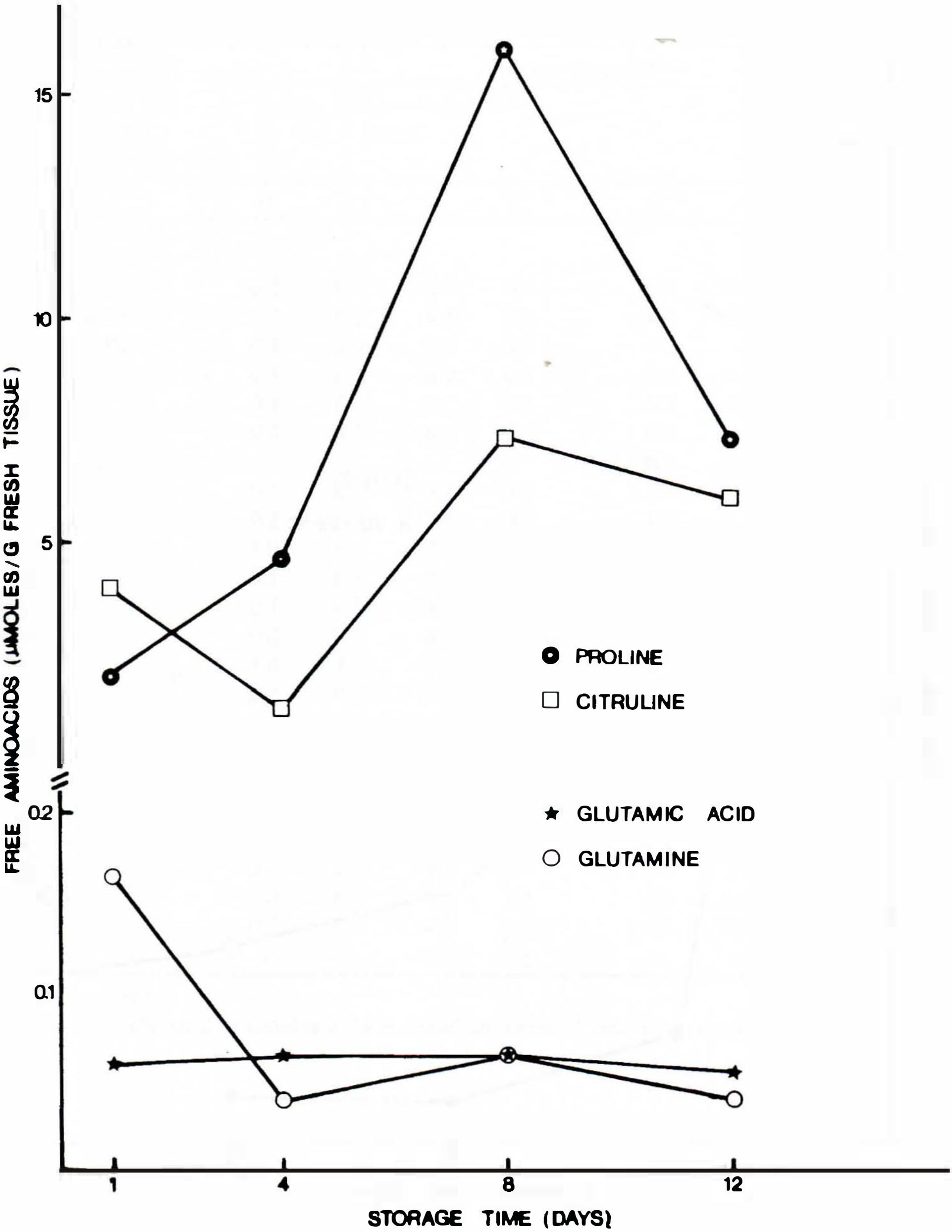

Fig. 2.—Changes in the levels of proline, citrulline, glutamic acid and glutamine in mesocarp of cherimoya fruits during ripening at 20-22 °C and 85% relative humidity.

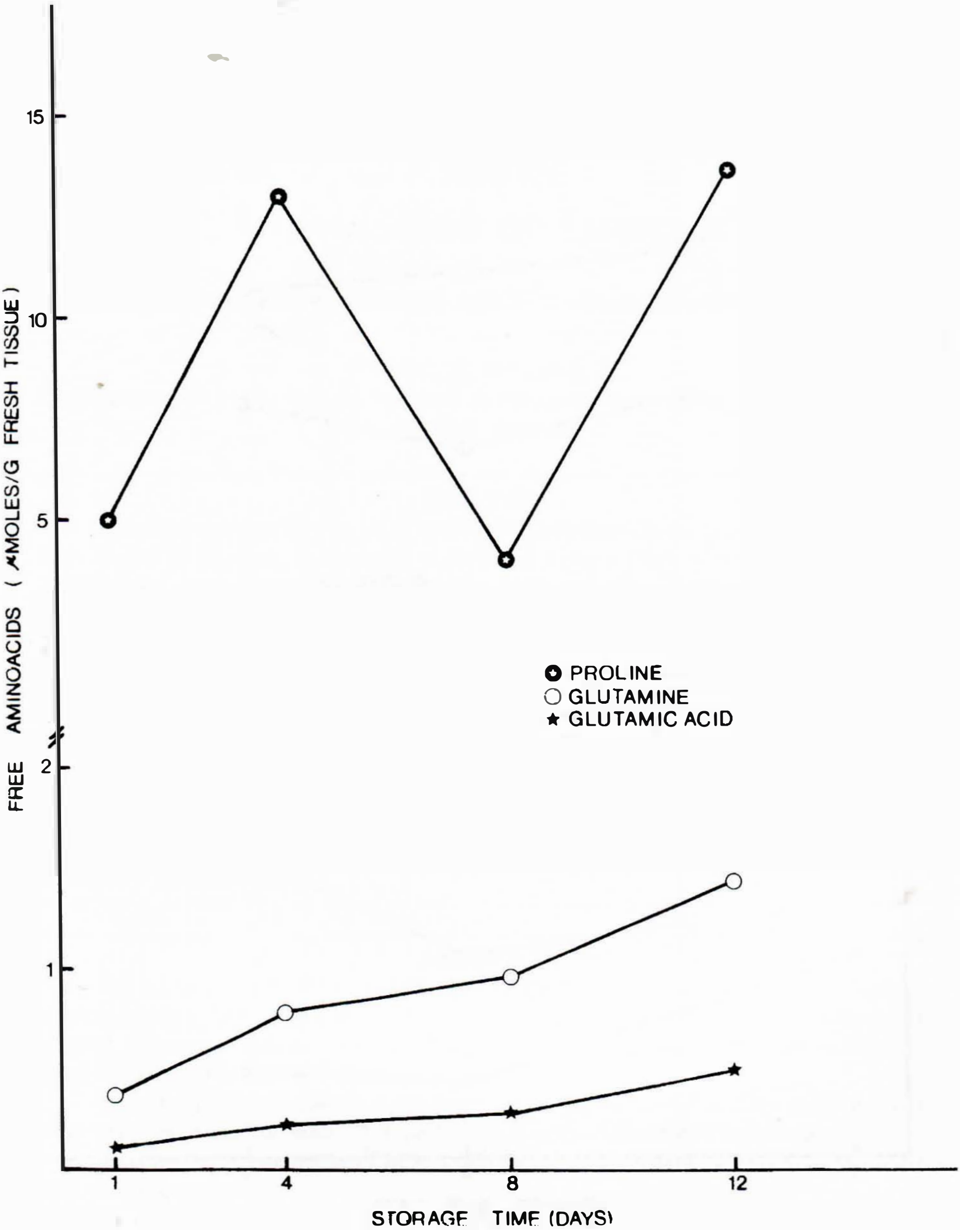

Fig. 3.—Changes in the concentrations of proline, glutamic acid and glutamine in epicarp of cherimoya fruits during ripening at 20-22 °C and 85% relative humidity.

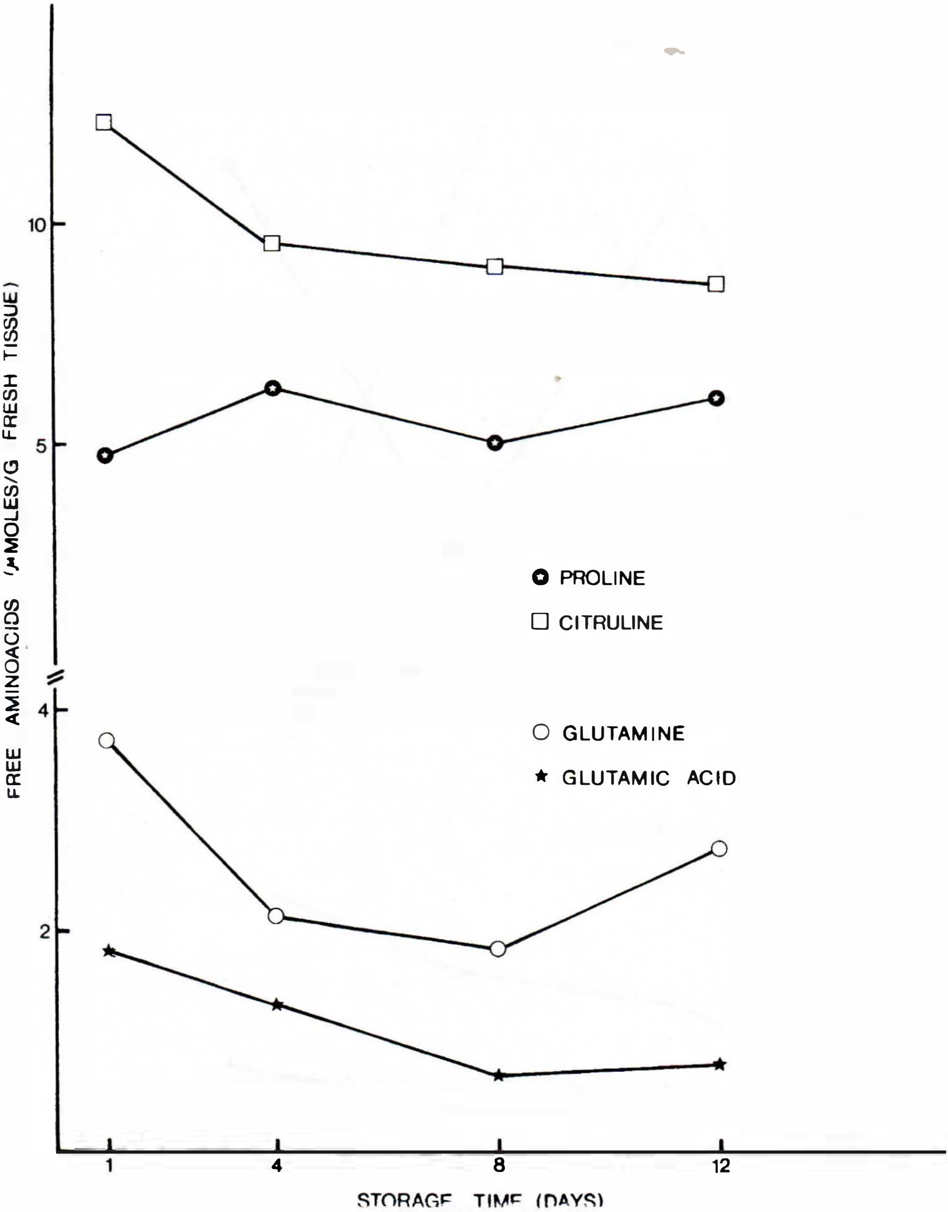

Fig. 4.—Concentrations of proline, citrulline, glutamic acid and glutamine in mesocarp of cherimoya fruits during ripening at 11 °C and 85% relative humidity.