Changes in the content of free amino acids in cherimoya fruits at various ripening temperatures

Cambios en el contenido de aminoácidos libres en frutos de chirimoyo a distintas temperaturas de maduración

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RESUMEN

Las variaciones en el contenido de aminoácidos libres en frutos durante la maduración es un tema de interés debido a la relación de estos compuestos con su olor, sabor y estructura. Este estudio se ha diseñado para determinar los cambios en aminoácidos libres durante la maduración de los frutos del chirimoyo *(Annona cherimolia* var. Fino de Jete), evaluando la influencia de la temperatura de almacenamiento después de la recolección. Extractos metanólicos de epicarpio y mesocarpio del fruto se usaron para la cuantificación de los aminoácidos libres, los cuales fueron analizados por cromatografía de intercambio iónico. Citrulina y prolina se detectaron en cantidades relativamente altas. Glutamato, glutamine, aspartato, asparragina y los no proteinogenéticos taurina, ácido γ-aminobutíricoy ácido α-aminoadípico fueron encontrados en concentraciones que variaron entre 2-6% del total del contenido de aminoácidos libres. La maduración incrementó el contenido de aminoácidos, sobre todo de prolina, citrulina y glutamato, cuando los chirimoyos se almacenaron a 20 °C o 8 °C. Este incremento no ocurrió cuando la temperatura de almacenamiento se mantuvo a 4 °C, sugiriendo que el daño por frío afecta marcadamente a la vía de síntesis de la prolina a partir de glutamato.

Palabras clave: Aminoácidos. Annonacea. Chirimoya. Maduración.

ABSTRACT

The variations in the content of free amino acids in fruits during ripening is a matter of interest since these compounds are related to their flavour, taste and structure. This study was designed to determine the changes of free amino acids in ripening cherimoya fruits *(Annona cherimolia* var. Fino de Jete), evaluating the influence of the storage temperature after harvesting. Methanolic fruit extracts of epicarp and mesocarp were used for quantification of free amino acids which were analyzed by ion-exchange chromatography. Citrulline and proline were detected in relatively high amounts. Glutamate, glutamine, aspartate, asparagine and the non proteinogenic taurine, γ-aminobutyric and γ-aminoadipic acids were found in concentrations that varied between 2-6% of the total free amino acids content. Ripening increased the contents of amino acids, mainly proline,

citrulline and glutamate when cherimoya storage at 20 °C or 80 °C. These increases did not occur when the storage temperature was maintained at 4 °C suggesting that chilling injury markedly affects the synthesis pathway of proline from glutamate.
Key words: Amino acids. Annonaceae. Cherimoya. Ripening.

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INTRODUCTION

Cherimoya fruit is one of the richest sources of free amino acids, accounting for 10.4% of fruit dry weight. (1, 2). In the last few years, a great deal of interest has been centered on the free amino acid content of some fruits (3-6). Ventura *et al.* (7) have examined the sugar apple *Annona squamosa*, which presents the unusual and interesting metabolical characteristic that γ-aminobutyric acid and citrulline predominate among the total amino acids, including arginine and ornithine. In the soursop *(Annona muricata)* proline and γ-aminobutyric acid are the major amino acids, together with nine others which appear in smaller amounts (7). Recently Touche *et al.* have reported that *Annona* cf. *crassiflora* and *Annona paludosa* are characterized by a high content of basic amino acids, particularly histidine, lysine, arginine and ornithine. (3).

The variations in the content of free amino acids in relation to metabolic changes occurring during growth and ripening, processes that are of fundamental importance in controlling the post-harvest behaviour of fruits, have received litle attention. At present there are no data concerning the qualitative and quantitative patterns of free amino acids in ripenig cherimoya fruits.

The aim of this study was to determine the changes in the content of free amino acids of ripening cherimoya fruits after harvest at the green-ripe stage. To evaluate the influence of chilling injury, cherimoya fruits were stored at 8 °C and 4 °C and the changes in the free amino acid patterns were compared with those obtained in fruits stored at 20 °C.

MATERIALS AND METHODS

Plant material

Uniform weight (500 + 50 g) cherimoya fruits *(Annona cherimolia Mill)* of the "Fino de Jete" variety were harvested at the green-ripe stage and subsequently ripened under one of the following temperatures: 20 °C, 8 °C or 4 °C, and at 85% relative humidity (RH).

The fruits were stored for 12 to 40 days depending on the ripening temperature. Sampling of cherimoyas for amino acid analysis was done at 4 day intervals during ripening.

Amino acid analysis

The fruits were washed with tap water and dried with filter paper. Mesocarp was separated of the epicarp by a stainless steel spatula and seeds were removed. Each tissue was cut into small pieces and weighed. Samples homogenization were carried out separately with approximately 100 g of mesocarp and 50 g of epicarp. Two volumes of 80% methanol in water containing 0.2% sodium disulphite by epicarp or mesocarp weight were added. Suspensions were obtained by disintegration using a Sorvall Omnimixer homogenizer for 5 min. The suspensions were filtered twice through four layers of surgical gauze. The methanolic extract was centrifuged at 1,200 g and vacuum concentrated at 40-50 °C.

Final volumes for epicarp and mesocarp extracts were respectively adjusted to 100 ml. and 50 ml Samples for amino acid analysis were diluted five times with a buffer pH 2.1, containing 250 nmoles of norleucine as internal standard.

Amino acids were determined by ion-exchange chromatography using a Chromaspeck-J-180 automatic amino acid analyzer as described previously (8).

RESULTS

Table 1, 2 and 3 respectively show the contents of mesocarp and epicarp free amino acids of cherimoya fruits ripened at 20 °C, 8 °C, and 4 °C after harvest at the green-ripe stage.

The major free amino acids detected in both tissues were citrulline and proline. The concentration of citrulline accounted for 11-65% and 12-44% of total free amino acids in epicarp and mesocarp respectively. The relative contents of proline varied between 11-58% and 16-60%.

Acidic amino acids and their amides as well as serine were also detected in relatively high amounts (2-6%). On the contrary, aromatic and basic amino acids, except ornithine, were present in low amounts. Ornithine represented 2-20% of total free amino acids both in epicarp and mesocarp of cherimoya fruits.

Branched chain and neutral aliphatic amino acids were always present in the fruits but their levels were very low. Threonine and hydroxyproline were also detected in low amounts.

The non-proteinogenic amino acids, taurine, γ-aminobutyric and α-aminoadipic were regularly found in quantities that varied between 2-5% of the total free

TABLE 1.—Mesocarp and epicarp free amino acid contents of cherimoya fruits ripened at 20 °C-22 °C and 85% relative humidity after harvesting at the green-ripe stage *

	Epicarp				*Mesocarp*			
Amino acid	*Day after harvesting*				*Days after harvesting*			
	1	*4*	*8*	*12*	*1*	*4*	*8*	*12*
TAU	0.42	1.38	0.39	1.33	0.51	0.11	0.45	0.52
α-AA	0.42	0.52	0.70	1.03	0.17	0.21	0.60	0.45
γ-AB	—	—	—	—	0.33	0.19	0.68	0.52
ASP	0.59	0.31	0.14	0.30	0.05	0.15	0.18	0.12
THR	0.21	0.19	0.31	0.48	0.12	0.08	0.17	0.14
SER	0.47	0.26	0.22	0.46	0.19	0.10	0.16	0.16
ASN	0.82	0.41	0.65	0.88	—	—	—	—
GLU	0.11	0.28	0.32	0.50	0.16	0.04	0.07	0.06
GLN	0.38	0.71	0.95	1.43	0.06	0.07	0.07	0.04
PRO	4.90	12.60	2.41	13.42	1.91	4.90	17.12	7.20
OH-PRO	0.17	0.21	0.36	0.56	0.09	0.15	0.16	0.18
GLY	0.11	0.08	0.16	0.10	0.06	0.08	0.18	0.15
ALA	0.10	0.19	0.21	0.17	0.21	0.17	0.14	0.11
CIT	11.82	14.33	15.83	14.21	3.81	1.72	7.30	6.00
VAL	0.25	0.22	0.58	0.58	0.11	0.06	0.23	0.20
ILEU	—	—	0.45	0.23	—	—	—	—
LEU	—	—	0.25	0.20	0.04	0.04	0.06	0.11
TYR	0.06	0.15	0.11	0.25	0.07	0.08	0.11	0.15
PHE	0.27	0.10	0.32	0.62	0.17	0.17	0.14	0.10
TRP	0.08	0.72	0.14	0.23	0.03	0.08	0.13	0.05
HIS	—	—	—	—	0.06	0.08	0.10	0.06
ORN	1.21	1.84	1.60	2.21	0.90	0.45	1.05	1.30
LYS	0.21	0.28	0.32	0.36	0.15	0.07	0.15	0.18
ARG	—	—	—	—	0.00	0.01	0.02	0.03

— = Not detected.

* Results are expressed as mean of four determinations in μmoles of free amino acids per g of fresh tissue.

amino acids. Citrulline was regularly detected in higher concentrations in epicarp than in mesocarp with independence of the ripening temperature. Proline was also found elevated in epicarp with the increases more evident at 8 °C and 4 °C. At 20 °C the concentration of glutamete was increased in the epicarp compared to that of the mesocarp but it did not present significant differences between both tissues at 8 °C and 4 °C.

The concentrations of citrulline and proline were increased at 8 °C and decreased at 4 °C in relation to those of 20 °C in both epicarp and mesocarp, and glutamine and serine were found at higher levels at 8 °C and 4 °C than at 20 °C.

TABLE 2.—Mesocarp and epicarp free amino acid contents of cherimoya fruits ripened at 8 °C and 85% relative humidity after harvesting at the green-ripe stage *

Amino acid	*Epicarp*				*Mesocarp*			
	Day after harvesting				*Days after harvesting*			
	1	*8*	*16*	*20*	*1*	*8*	*16*	*20*
TAU	0.43	0.66	0.53	0.79	0.57	0.41	0.40	0.42
α-AA	1.21	1.37	0.82	3.08	1.21	0.85	0.92	2.05
γ-AB	—	—	—	—	0.65	0.33	0.74	0.58
ASP	0.46	1.05	0.56	0.81	0.47	0.32	0.44	0.37
THR	0.22	0.58	0.31	0.75	0.37	0.33	0.54	0.60
SER	0.59	1.05	0.64	1.24	0.58	0.45	0.53	0.67
ASN	1.16	1.46	0.45	0.62	2.05	1.67	0.61	1.05
GLU	3.20	2.90	1.39	2.03	1.87	1.02	0.80	0.82
GLN	2.10	1.91	1.10	2.10	6.42	2.97	1.95	2.25
PRO	23.95	22.71	25.42	24.82	38.05	15.21	19.25	9.75
OH-PRO	0.31	0.24	0.32	0.38	0.23	0.46	0.69	0.32
GLY	0.17	0.22	0.15	0.10	0.02	0.01	0.05	0.07
ALA	0.21	0.34	0.15	0.17	0.32	0.41	0.20	0.18
CIT	20.92	19.21	15.13	24.08	8.72	11.33	7.40	7.52
VAL	—	—	—	—	0.32	0.47	—	—
ILEU	0.26	0.25	0.19	0.25	—	0.30	0.42	0.71
LEU	0.05	0.04	0.04	0.08	0.07	0.17	0.06	0.07
TYR	—	—	—	—	0.02	0.05	0.03	0.04
PHE	0.10	0.18	0.15	0.14	0.03	0.17	0.08	0.11
TRP	—	—	—	—	0.02	0.12	0.03	0.06
HIS	0.19	0.15	0.24	0.14	0.05	0.10	0.12	0.21
ORN	1.35	2.95	1.25	0.82	1.21	2.05	1.08	4.10
LYS	—	—	—	—	0.60	0.71	0.23	0.35
ARG	0.51	0.50	0.40	0.45	0.16	0.23	0.24	0.31

— = Not detected.

* Results are expressed as mean of four determinations in µmoles of free amino acids per g of fresh tissue.

DISCUSSION

The amino acid profile of cherimoya during ripening after harvest at the green-ripe stage was characterized by a high content of citrulline and proline and moderate concentrations of ornithine, glutamate, aspartate, glutamine and asparagine and low levels of branched chain aromatic and basic amino acids.

Some tropical Annonaceae contain high levels of basic amino acids in the leaves as well as in the fruits, citrulline and ornithine being the most frequently isolated (3). Futhermore, sugar apple also contain high levels of citrulline (6) and many other fruits not related to cherimoyas are also characterized by the presence of high concentrations of basic amino acids (5, 6, 9, 10). Proline is

TABLE 3.—Mesocarp and epicarp free amino acid contents of cherimoya fruits ripened at 4 °C and 85% relative humidity after harvesting at the green-ripe stage *

	Epicarp				*Mesocarp*			
Amino acid	*Day after harvesting*				*Days after harvesting*			
	1	*12*	*24*	*32*	*1*	*12*	*24*	*32*
TAU	0.95	0.48	0.98	0.83	0.26	0.39	0.63	0.03
α-AA	0.75	0.23	1.62	1.21	0.44	0.20	0.65	0.24
γ-AB	0.45	0.40	0.82	2.71	0.16	0.44	0.55	0.42
ASP	0.98	0.70	2.15	1.06	0.97	0.81	0.75	0.31
THR	0.17	0.10	0.38	0.44	0.26	0.13	0.29	0.10
SER	0.68	0.35	1.41	1.10	0.68	0.44	0.78	0.39
ASN	0.27	1.12	0.61	0.50	0.23	0.13	0.23	0.22
GLU	1.40	0.91	3.05	1.10	1.87	1.39	1.71	0.78
GLN	2.01	3.20	1.42	1.56	1.88	0.80	1.30	1.07
PRO	1.22	0.32	3.10	2.58	0.88	0.37	0.65	1.17
OH-PRO	0.98	0.65	1.85	2.02	0.26	0.37	1.07	0.10
GLY	0.12	0.17	0.08	0.15	0.26	0.05	0.10	0.11
ALA	0.57	0.25	0.93	1.04	0.24	0.29	0.58	0.28
CIT	11.20	6.62	3.95	19.35	2.48	3.67	2.36	5.72
VAL	0.12	0.17	0.22	0.34	0.10	0.03	0.05	0.05
ILEU	0.25	0.26	0.29	0.03	0.05	0.15	0.13	0.06
LEU	0.05	0.06	0.06	0.06	0.02	0.02	0.03	0.09
TYR	0.13	0.28	0.21	0.20	0.10	0.05	0.15	0.04
PHE	0.61	0.07	0.52	0.38	0.12	0.05	0.06	0.22
TRP	0.27	0.12	0.26	0.14	0.02	0.04	0.02	0.02
HIS	0.01	0.15	0.05	0.06	0.01	0.04	0.07	0.09
ORN	—	—	—	—	0.17	2.65	0.84	0.94
LYS	—	—	—	—	0.04	0.05	0.04	0.04
ARG	0.05	0.07	0.25	0.06	0.04	0.00	0.04	0.32

— = Not detected.

* Results are expressed as mean of four determination in μmoles of free amino acids per g of fresh tissue.

usually present in fruits during the ripening process (11) and the soursop, a kind of cherimoya, contains this amino acid at the highest levels among the studied Annonaceae (7). γ-aminobutyric acid was present in cherimoya but its level was only more than 2% of the total amino acids in contrast to the results reported for other Annonaceae (6).

The ripening process has been observed to be intimately associated with the metabolism of arginine, proline and glutamic acid. The proline level in particular being increased at the height of the ripening process (11).

In our study proline and citrulline as well as glutamate increased quickly after harvest in epicarp and mesocarp of cherimoya fruits. The increase in

proline was not correlated with a decrease in arginine and glutamate as it has been described in grapes (6). However, Pool *et al.* (12) have recorded a rise in the levels of both arginine and proline during long term storage.

The epicarp of cherimoya was richer than mesocarp in the concentration of citrulline, proline and glutamate. This fact may be related to a more active synthesis pathway in the first tissue since proline is one of the principal compounds in the maintenance of the plant cell wall structure.

When the ripening process of the cherimoyas was carried out at 8 °C, we obtained the highest concentrations for citrulline, glutamate and proline, both for epicarp and mesocarp. It seems that decreasing in temperature of ripening is related to an increase in the activity of the enzymic systems to produce more proline. However, chilling injury occurring at 4 °C markedly decreased the levels of citrulline, glutamate and proline both in epicarp and mesocarp. From the data obtained in this study we may conclude that the ripening process and the beginning of the fruit senescence in cherimoya are correlated with increases in the levels of proline, citruline and glutamate, which appears to be metabolically associated, and that chilling injury, considerably affects the accumulation of amino acids that normally occur during the ripening period.

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