

Vitamins in fruits and vegetables of the Amazon. 1. Methods for the determination of β -carotene, tocopherol and ascorbic acid with high performance liquid chromatography (HPLC).

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Summary

At the beginning of an inventory of the chemical composition of regional fruits and vegetables of the Amazon, analytical methods were adapted for the high performance liquid chromatography (HPLC) determination of pro. vitamin A (β -carotene), vitamin C (ascorbic acid) and vitamin E (tocopherol). The first analyses indicate as excellent sources of β -carotene, *Mauritia flexuosa* L., *Astrocaryum tucuma* Mart. and *Cucurbita pepo* L.; of ascorbic acid *Theobroma grandiflorum* (Cullis ex Spreng.) Schum. and *Gnetum paniculatum* Spr. ex Benth. and of vitamin E, *Mauritia flexuosa* L. and *Euterpe oleracea* Mart.

INTRODUCTION

The Amazon region has the potential to deliver a variety of regional fruits and vegetables. Cavalcante (1976) described a number of edible fruits (128) from this area. Most have their botanical origin in this region, but until now only a part of them are commonly known and cultivated for commercial use. These many interesting Amazonian fruits are rarely used for human consumption, and knowledge of their chemical composition and nutritional value is very limited. With regard to the chemical composition of the majority of the regional species of fruits and vegetables in the northern Brazil, there has been no literature available for the

past ten years. On the other hand, the availability of more, and cheaper, fruits and vegetables that are rich in vitamins and mineral salts, could be an inducement to increased consumption, and consequently improve the nutritional habits of the Amazon people. Recent investigations (Mello Amorozo, 1980; Shrimpton & Giugliano, 1979) show that the poor people of Manaus suffer from a lack of vitamin A, thiamine and riboflavine and some mineral salts. Therefore, an inventory of nutrients is planned, especially of the vitamins contained in fruits and vegetables of the Amazon, to act as an important basis for providing a knowledge of available regional (and therefore cheaper) foods of high nutritional value.

The few data of vitamins from foods published for use in Latin America and Brazil (McDowell et al., 1974; Sizereith & Jardin, 1977), need to be revised. In recent years methods for the determination of vitamins have been essentially improved by introduction of gas chromatographic (GLC) and high performance liquid chromatographic (HPLC) methods. The traditional methods are either expensive and time consuming biological methods, or unspecific titulations and color reactions, or based on the little sensitive thin layer chromatography. The HPLC based separations have some real advantages: the samples need only little pretrea-

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tments before the injection; the total analysis time is short and unlike GC methods there is generally no need for derivatization. Therefore, the risk for light and oxygen induced degradation of vitamins during the clean up is reduced and consequently, the results are more accurate.

The following methods are adaptations and improvements of other recently published works with the aim of presenting quick, exact and the simplest possible methods for the HPLC determination of vitamins in tropical fruits and vegetables.

EXPERIMENTAL

β - Carotene

a) Extraction (Rückermann & Ranfft, 1978): Weigh up to 10g of the edible part of the sample and place in a mixer along with 100ml of cold ethanol (5°C), add about 50mg of butylhydroxyanisole and 2g of CaCO₃ and mix thoroughly. Transfer to a volumetric, brown colored flask of 500ml, wash the residues from the mixer with cold ethanol, and place in the flask. Add exactly 20ml of light petroleum (20°C), shake thoroughly and refrigerate for at least 10 minutes. Then add aqueous solution with 1.5% NaCl until the liquid level is a little bit under the neck of the flask. Shake thoroughly. Soon after there will appear a separation into two layers. Add more of the NaCl solution until the upper organic layer is in the neck of the flask and easily removable for injection into the HPLC system.

b) Chromatographic system. Column: 25cm x 4mm, filled with ODS C18, 7 μ m equipped with a precolumn (filled with the same material). Mobile phase: acetone : water (88:12), 1ml/min. The peaks are detected by their absorption at

450nm. The precolumn is changed after each 50 injections.

Tocopherol

a) Sample preparation. Oils are only diluted (1: 10) with n-hexane: isopropanol (98.5:1.5) in brown colored flasks (Carpenter, 1979). Fruits and vegetables are extracted with light petroleum like that described for β -carotene.

b) Chromatographic system. Column: 30cm x 2mm filled with μ -Porasil. Mobile phase: n-hexane : isopropanol (98.5 : 1.5), 1ml/min. The peaks are detected by their absorption at 295nm. The detection is more sensitive and specific when using a spectrofluorimeter with the excitation wavelength set at 298nm and the emission wavelength set at 330nm. After approximately 30 injections the column needs a treatment with methanol for 30 minutes. Because n-hexane and methanol are not miscible, acetone is used as an intermediate solvent. Before the next analysis let the system reequilibrate with the mobile phase for at least 30 minutes.

Ascorbic acid

a) Preparation of the solutions. Solution 1: add 1 flask of the ion pair reagent A (Waters) to 1l of bidest. Water resulting in a 0,005 molar solution of tetrabutylammonium phosphate, shake and filtrate through a membrane filter of 0.45 μ and adjust with 0.1N HCl to pH 4. Solution 2: add 1 flask of the ion pair reagent A (Waters) to 1l of methanol (HPLC quality), shake and filtrate through a membrane filter of 0.45 μ . Solution 3: 1% oxalic acid in bidest (Water). Solution 4 (standard solution of ascorbic acid): add 1 to 10mg of ascorbic acid pa. (Merck) to 50ml of solution 3.

b) Sample preparation. Homogenize 10 to 30g or more of the sample with 50ml of solution 3, refrigerate for 15 minutes, centrifugate with 3000 rot/min. in a closed tube, filtrate through a microfilter before injection.

c) Chromatographic system. Column: 25cm x 4 mm filled with ODS C18, 7 μ m equipped with a precolumn (filled with the same material). Mobile phase: solution 1/solution 2 (75: 25). The peaks are detected by their absorption at 250nm.

DISCUSSION

β - Carotene

β -carotene is the most important vitamer with the highest biological provitamin A activity. In plants there may be a lot of other carotenoids with considerable activities (Figure 1 and Table 1).

Methods based on HPLC are able to separate all relevant compounds, on principle this can be done by direct phase or reversed phase HPLC. The methods (Fiksdahl, Mortensen & Liaaen-Jensen, 1978; Reeder & Park, 1975) based upon direct

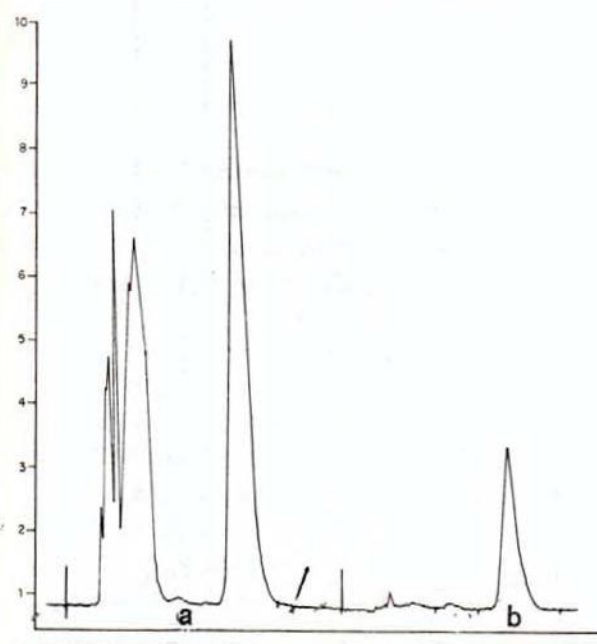


Figure 1 — Chromatogram of (a) extract of *Celosea argentea*; (b) standard of B-carotene

Table 1. Biological activity of the following vitamers (Schormüller, 1974)

Provitamin A	
β - carotene	100
cryptoxanthin	57
β -apo- γ -carotenal	56
neo-B- β -carotene	53
α -carotene	53
γ -carotene	42
neo-U- β -carotene	38
Vitamin E	
α -tocopherol	100
β -tocopherol	40
γ -tocopherol	30
δ -tocopherol	1
Vitamin C	
ascorbic acid	100
dehydroascorbic acid	100

phase separation (polar stationary and unpolar mobile phase) suffer from the problem that after injection of samples with a polar matrix, the columns soon get dirty and need a prolonged time to reequilibrate after cleaning the column with polar solvents and simply after using a solvent gradient. Therefore today the reversed phase HPLC is generally preferred, about 80% of the total HPLC analyses are put through on reversed phase systems.

For the reversed phase separation of carotenoids a mixture at chloroform and acetonitrile is recommended as mobile phase (Zakaria & Simpson, 1979). But as acetonitrile is expensive we tried the separation with a mobile phase of a mixture of water and acetone in accordance with Langer (1976). The number of carotenoids occurring in an individual sample can make it necessary to change the percentage composition of the solvents in order to obtain the best separation. For the beginning it was restricted to determining the content of β -carotene. For a future pu-

blification the identification of the occurring carotenoids in order to calculate the exact biological provitamin A value of each sample is being planned. In some fruits, for instance in *Endopleura uchi* (Huber) Cuat. and *Byrsonima crassifolia* H.B.K. no β -carotene was found, but rather relevant amounts of other unidentified carotenoids.

After prolonged use the precolumn gets dirty, resulting in the degradation of β -carotene which leads to a double peak for the standard β -carotene accompanied by a pressure rise in the column. To avoid this, change the precolumn routinely after approximately 50 injections. Moreover the lifetime of the column gets extended considerably. Depending on the sample it may be necessary to include a saponification step before the HPLC to avoid interferences between carotenoids and fat materials on the column.

For the method described the following parameters were determined: The detection limit was 3ng of β -carotene corresponding 12 μ g/100g in the sample. If the carotene content is smaller than that value, it is possible to use a larger quantity of the sample for the analysis in order to reduce the detection limit. The detector worked linear up to 4 μ g of β -carotene. The addition of β -carotene to *Theobroma grandiflorum* solution resulted in a good recovery of 97.8%.

The first results were obtained by testing the method. They are presented in Table 2. Satisfactory Contents of β -carotene in the fruits of *Astrocaryum tucuma*, *Mauritia flexuosa* and *Cucurbita pepo* (regional species) were found. These values are high compared to carrots which are considered as rich in β -carotene with 8.1mg/100g.

Tocopherol

The vitamin activity of the individual vitamers is different (Table 1). Therefore it is important to separate them in

order to calculate the real vitamin E value of each sample. The biological activity of the tocotrienols has not yet been well examined. In any case it is considered less compared with the respective tocopherols. Therefore it was not taken into consideration.

Some recent publications (Carpenter, 1979; Rückermann & Ranfft, 1978 and Gertz & Herrmann, 1982) show that the separation of tocopherol takes place very well with direct phase HPLC. We took the mentioned drawbacks into the bargain and used a mixture of n-hexane: isopropanol (98.5: 1.5) as mobile phase. In this way all the tocopherols are separated and eluted within 9 minutes. The absorption coefficients of the tocopherols are quasi equal, therefore the quantitative calculations were based upon a standard solution of α -tocopherol. Addition of α -tocopherol to a sample of *Mauritia flexuosa* resulted in a recovery of 87.5%.

Figure 2 shows that this simple and rapid method is able to separate the tocopherols in natural products well.

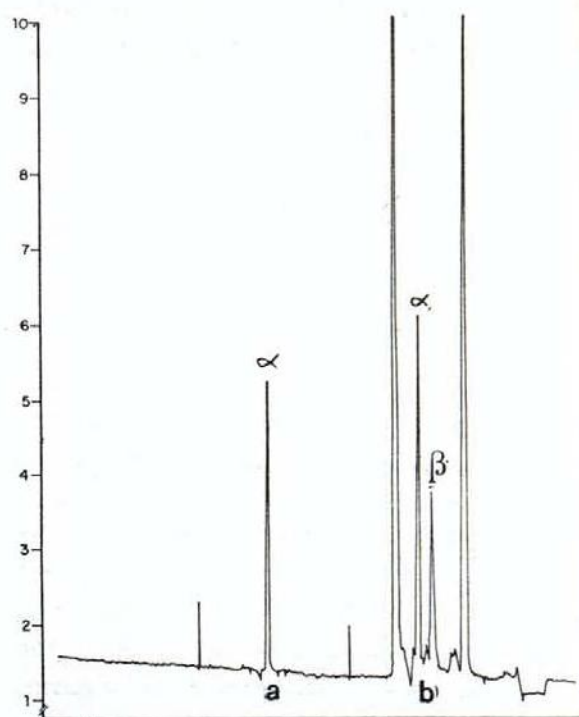


Figure 2 — Chromatogram of (a) standard of α -tocopherol; (b) extract of "dôce de buriti"

Table 2. Vitamins in selected tropical fruits and vegetables

Species	Ascorbic acid	β -Carotene	Tocopherol
	mg/100 g		
<i>Platonia insignis</i> (bacuri)	1.3		0.3 (α)
<i>Rheedia acuminata</i> (bacuri de espinho)	1.6		
<i>Rheedia brasiliensis</i> (bácuri selvagem)	34.9		1.9 (α)
<i>Couma guianensis</i> (sôrva)	1.2		
<i>Theobroma grandiflorum</i> (cupuaçu)	38.3	0.03	
<i>Byrsonima crassifolia</i> (murici)	8.1		
<i>Spondias lutea</i> (taperebá)	17.2		
<i>Inga fascistipula</i> (ingá chichica)	1.0		
<i>Passiflora nitida</i> (maracujá do mato)	6.7		
<i>Calathea allouia</i> (ariá)	2.8		
<i>Gnetum paniculatum</i> (ituá)	62.9		
<i>Mauritia flexuosa</i> (buriti)		12.0	18.4 (α) 13.7 (β) 0.2 (γ)
Doce de buriti (commercial sweet preparation)	4.9		25.0 (α) 12.0 (β) Trace (γ)
<i>Euterpe oleracea</i> (açai)			7.7 (α) 0.8 (β) 2.0 (γ)
Suco de açai (commercial juice)			6.0 - 9.3 (α) 0.5 - 1.2 (β) 2.0 - 2.1 (γ)
<i>Celosea argentea</i> (celosea)			4.9 (α) 0.3 (β) 2.8 (δ)
<i>Astrocaryum tucuma</i> (tucumã)		13.6	
<i>Cucurbita maxima</i> (jurumum caboclo)		6.2	
<i>Cucurbita pepo</i> (jurumum de leite)		15.5	
Boga-boga (a species of Cucurbitaceae)	7.3		

The necessary daily intake for adults is 10 to 25mg of α -tocopherol. Thus the fruits of *Mauritia flexuosa* (local name: buriti) are easily able to meet this need (Table 2). According to our analyses the processed food "doce de buriti", which is common in the environs of Bahia, did not lose notable amounts of tocopherols during the processing. The commercial juice of *Euterpe oleracea* can also be con-

sidered as a good source of tocopherols either.

Ascorbic acid

Ascorbic acid (AA) and its direct oxidation product, dehydroascorbic acid have, the same efficiency as vitamin C. But in intact fruits and vegetables the total amount of vitamin C is present as ascor-

bic acid, because the oxidation begins after the harvest in contact with the air. Therefore the determinations were limited to the original AA.

Recent publications about the HPLC determination of AA use reversed phase systems and some authors employ aqueous buffer solutions as mobile phase (Rückermann, 1980). But AA is a relative polar substance and thus it elutes very rapidly in such systems. To avoid coelution with other polar compounds in food samples, we preferred to add an ion pair reagent to the mobile phase according to Wills, et al., (1977) who proposed this for the separation of pure watersoluble vitamins. The ion pair reagent tetrabutylammonium forms with AA a less polar com-

plex. This complex has more affinity to the stationary phase and therefore elutes later than ascorbic acid in aqueous buffers. The proposed method requires very little preparation. The danger of decomposition during the clean up is minimized because the total analysis time is very short. Figure 3 shows a typical determination of ascorbic acid. Some analyses were carried out (Table 2), which show that *Theobroma grandiflorum* and *Gnetum paniculatum* (local names: cupuaçú and ituá, respectively) are excellent sources of vitamin C. The latter could substitute potatoes in the tropical regions. Comparable to the lemon: 53mg AA/100g.

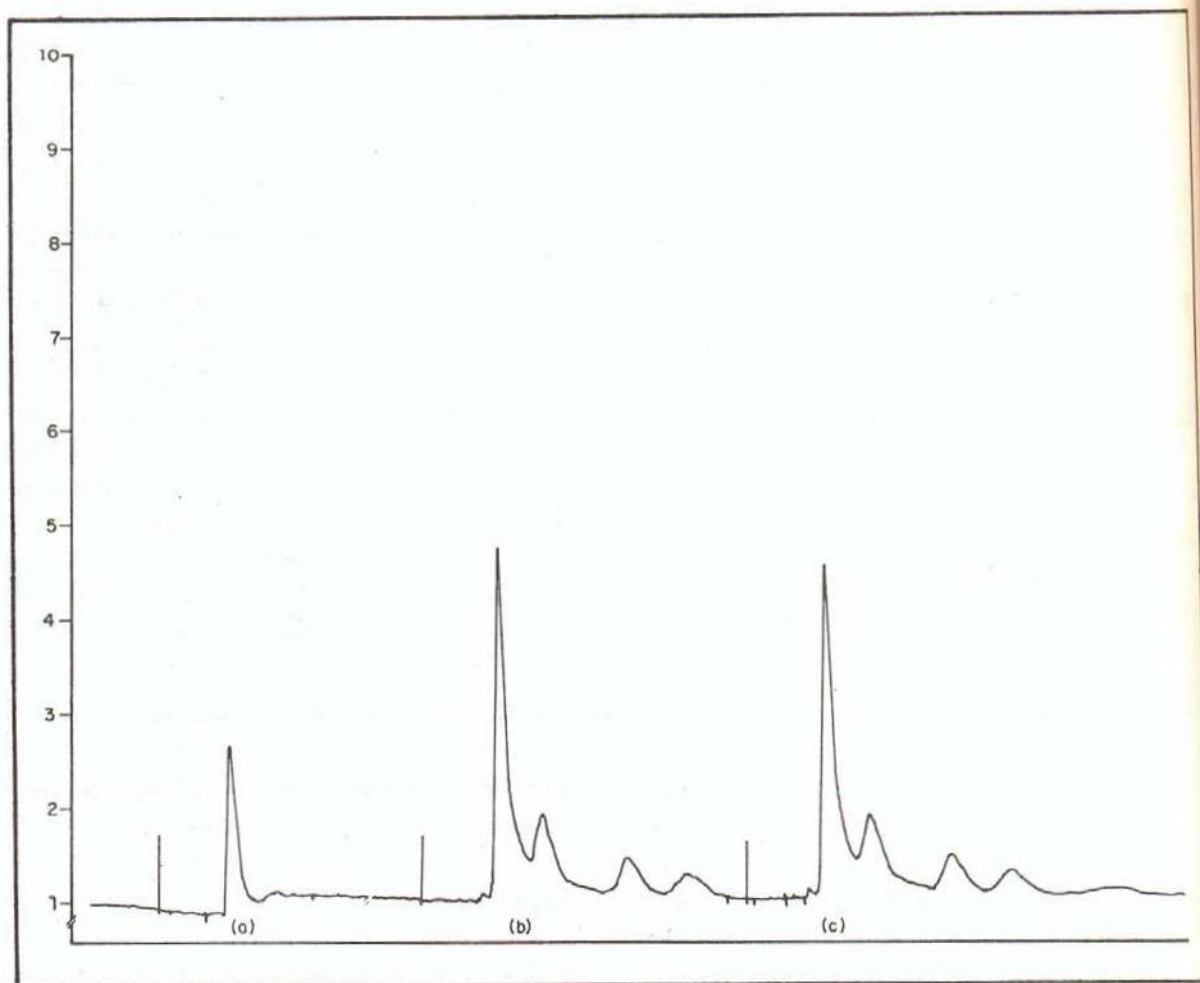


Figure 3 — Chromatogram of (a) standard of ascorbic acid; (b) and (c) extract of "ituá"

CONCLUSION

It has been shown that HPLC methods are useful for the determination of the vitamins tocopherol, ascorbic acid and β -carotene in fruits and vegetables. The first analyses carried out indicate that some uninvestigated regional fruits and vegetables of the Amazon have considerable amounts of vitamins. This work will continue in order to obtain more knowledge about the chemical composition and nutritional value of these tropical foods.

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Resumo

No começo de um levantamento da composição química de frutos e legumes da região amazônica, adaptou-se métodos analíticos para a determinação de pro-vitamina A (β -caroteno), vitamina C (ácido ascórbico) e vitamina E (tocoferol), através da cromatografia líquida de alta pressão (HPLC). As primeiras análises indicam como excelentes fontes de β -caroteno, *Mauritia flexuosa* L., *Astrocaryum tucuma* Mart., e *Cucurbita pepo* L., de ácido ascórbico, *Theobroma grandiflorum* (Culls ex Spreng.) e de vitamina E, *Mauritia flexuosa* L. e *Euterpe oleracea* Mart.

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