Food Chemistry **47** (1993) 87–92
Analytical Methods Section

The partition of ascorbic and dehydroascorbic acid in vitamin C-containing Guatemalan foods

Julieta Quán de Serrano,* Lillian de González & Noel W. Solomons

Center for Studies of Sensory Impairment, Aging and Metabolism, the Research Branch for the Guatemalan National Committee for the Blind and Deaf, Guatemala City, Guatemala

(Received 24 October 1989; revised version received 22 August 1992; accepted 28 August 1992)

The total vitamin C content, and its partition between L-ascorbic acid (L-AA) and L-dehydroascorbic acid (L-DHAA) was determined by a previously described robotic analytical procedure in nine Guatemalan fruits and vegetables prepared as customarily consumed. Total vitamin C levels agreed substantially with analyses performed over 25 years ago, but the L-AA/L-DHAA partition varied considerably from oranges and chayote, which had exclusively L-AA, to banana and tomato, which had predominantly L-DHAA. The chemical form of vitamin C may have implications for cataractogenesis, nitrosamine-related carcinogenesis, and iron absorption.

INTRODUCTION

Humans (*Homo sapiens*) are one of the rare species in nature dependent on external (dietary) sources of vitamin C. Ascorbic acid is required for the post-transcriptional hydroxylation of proline in the formation of the cross-linked collagen in connective tissues (Tuderman *et al.*, 1977). In the diet, vitamin C exists in the form of two principal vitamers: L-ascorbic acid (L-AA, reduced form) and L-dehydroascorbic acid (L-DHAA, oxidized form). Both have equivalent dietary antiscorbutic activity (Fox & Levy, 1936; Roe & Barnum, 1936).

A host of other putative health benefits have been ascribed to vitamin C. Among the most solidly established are enhanced bioavailability of iron (Monsen *et al.*, 1978), and preventing intragastric accumulation of nitrosamine (Kim *et al.*, 1982). For both of these functions, the chemical form of vitamin C is critical; the reducing properties of the L-AA form are essential for the respective reactions.

Recently, interest has developed in understanding how the oxidant/antioxidant properties of vitamin C influences the process of photo-oxidation of the human ocular lens that produces the opacification leading to cataract formation (Varma *et al.*, 1984; Blondin *et al.*, 1986; Chandra *et al.*, 1986; Blondin & Taylor, 1987). It is known that the lens and aqueous humor concentrate vitamin C from the plasma (Pirie & Van Heyningen, 1956; Chandra *et al.*, 1986). Most investigators feel that the net effect is a protective, antioxidant action of L-AA at the level of the lens. A dissenting view (Bensch *et al.*, 1985; Russell *et al.*, 1987) proposes that L-AA interacts with divalent metal ions to generate oxidizing radicals. This apparent connection of vitamin C with cataractogenesis led us to conduct a survey of recent vitamin C intake in 104 consecutive patients admitted for cataract extractions at the 'Dr Rodolfo Robles V.' Eye and Ear Hospital in Guatemala City (Barrantes de González & Solomons, 1987). This allowed us to identify which foods and beverages provided most of the dietary supply of the vitamin. We present here the results of the analysis of total vitamin C and its constituent vitamers in nine fruits and vegetables that contribute significantly to the intake of vitamin C in the Guatemalan diet.

MATERIALS AND METHODS

Samples

Nine foods were selected for analysis: whole oranges; orange juice; tangerine; tomato; banana; plantain; potato, cauliflower and chayote (also known as güisquil). The foods were purchased in three local urban markets in Guatemala City on a single day. With the exception of the oranges, three items from each group were purchased at each market site. Six oranges were bought to provide for both whole orange and juice. The foods were placed in ventilated plastic bags, coded, and packed in dry ice in a portable cooler, and shipped directly to Beltsville, Maryland, USA on the

* To whom correspondence should be addressed at: CeSSIAM, Hospital de Ojos y Oídos, 'Dr Rodolfo Robles V', Diagonal 21 y 19 Calle Zona 11, Guatemala City, Guatemala.

following day. In Maryland, they were stored frozen for an additional 40 h before they were analyzed.

The foods were analyzed in the form in which they are customarily consumed in Guatemala. Not all of the transported specimens were analyzed, but items were chosen randomly from the three-market assortment provided from Guatemala. The orange juice was prepared by squeezing. The orange, tangerine, tomato and banana were defrosted and analyzed raw. The cauliflower was cooked in boiling water for 15 min. The potato was placed in cold water and cooked at a moderate heat for 20 min. The chayote and plantain were placed in cold water and cooked at moderate heat for 30 min. Each of these conforms to the customary preparation for consumption in Guatemala.

In an additional experiment, cauliflower was purchased locally at a Maryland market and divided into three portions. Within 24 h one portion was prepared for analysis raw, while a second portion was cooked in the manner described above. The third portion was stored frozen for a week, and then defrosted, cooked and analyzed.

Solid samples were homogenized in a food processor (Robot Coupe). The amounts taken for analysis from the homogenized samples depended on the expected vitamin content. All samples were prepared, extracted and analyzed under yellow light.

Reagents

The reagents were the same as those used in the previous report (Vanderslice & Higgs, 1985). They included: ascorbic acid (Sigma, St Louis, MO, USA); dehydroascorbic acid (Tridom, New York, USA); pyrogallol (J. T. Baker, Phillipsburg, NJ, USA); mercuric chloride, citric acid, sodium citrate, ethylenediaminetetraacetic acid, methylene chloride and hexane (Fisher, Silver Spring, MD, USA). The water used in the preparations was processed by reverse osmosis and further purified with a Milli-Q system (Millipore, Bedford, MA, USA).

Apparatus

The same robotic procedure previously reported was used (Vanderslice & Higgs, 1985). It involved a Zymark robotic system (Hawk *et al.*, 1982) geared to an eight-step procedure from food sample preparation to injection into the high-performance liquid chromatograph. The separation and quantitation of vitamin C by HPLC was performed using an Aminex A-25 column.

Data analysis

The total vitamin C content for each item was obtained by HPLC and determined as the arithmetic sum of the L-AA and L-DHAA fractions for a given food. The total vitamin and vitamer contents were expressed as mg per 100 g of fresh (frozen) weight. For reference, the values published in the *Food Composition Tables for Use in Latin America* (Wu-Lueng & Flores, 1961) were used. If a range of variation was not provided in the food tables, limits of ±5% were used for comparison with the present analyses.

RESULTS

The amounts of L-AA and L-DHAA are shown in Fig. 1. A distinct variation in the relative proportion of the two vitamers is manifested in these data. The foods are arranged in descending order of percentage of L-AA and ascending order of L-DHAA content. Orange and chayote have 100% of their vitamin C

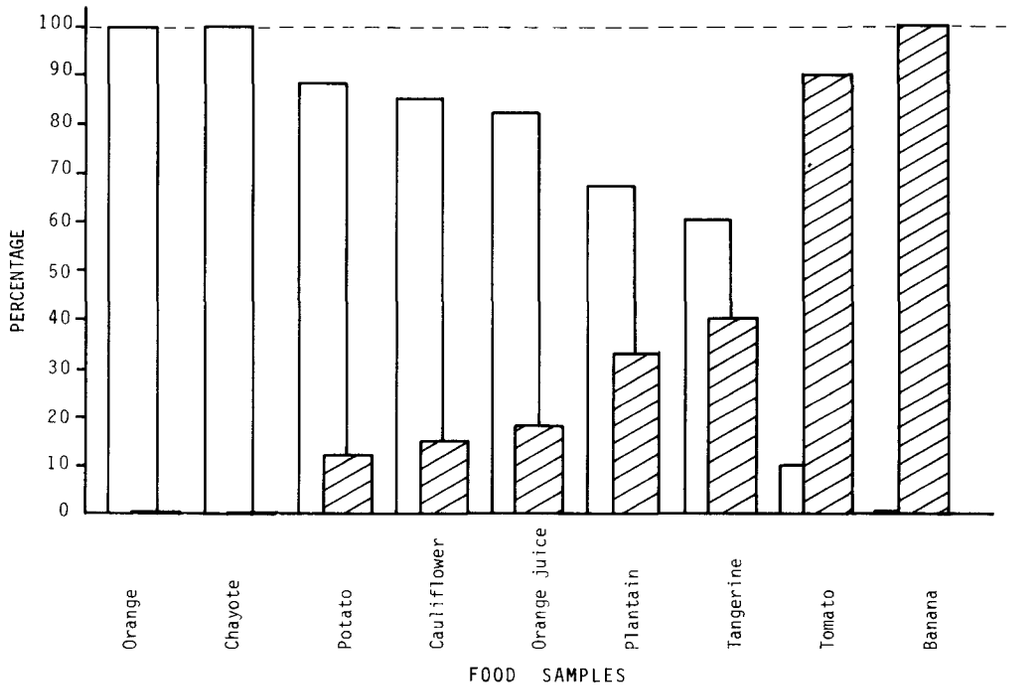

Fig. 1. Amounts of L-AA and L-DHAA determined. (□, L-AA; ▨, L-DHAA).

Table 1. Content of ascorbic acid and dehydroascorbic acid of Guatemalan food samples

Food	L-AA ($\mu g\ g^{-1}$)	L-DHAA ($\mu g\ g^{-1}$)
Banana (frozen)	—	10·7
Orange (frozen)	46·4	—
Orange juice	46·4	10·0
Tangerine (frozen)	22·6	15·0
Tomato (frozen)	2·2	19·0
Cauliflower (cooked)	27·3	4·7
Chayote or güisquil (cooked)	18·5	—
Potato (cooked)	32·8	4·3
Plantain (cooked)	11·3	5·5

in the reduced form, whereas all of the vitamin in banana is in the oxidized form and over 90% of the vitamin in tomatoes was found as L-DHAA. In all, six of eight items had more than 50% of their vitamin in the reduced form. It is interesting that squeezed orange juice had 20% less L-AA than the whole orange. This information is also summarized in Table 1.

Shown in Fig. 2 and Table 2 are the values for total vitamin C from the present study in relation to the

Table 2. Total content of vitamin C (ascorbic acid) of Guatemalan food samples and Latin American food composition table values

Food	Sample Total vitamin C (mg per 100 g)	Latin America Total vitamin C (mg per 100 g)
Banana	11	8–20
Orange	46	42
Orange juice	56	42
Tangerine	38	33
Tomato	21	23
Cauliflower	32	82
Chayote or güisquil	18	20
Potato	37	3–20
Plantain	17	20

range of vitamin C content for the corresponding eight items in the Latin American food tables.

Only cauliflower and potato from the present series differed markedly from the previously reported values. Cauliflower had less than half the expected value and potato had over twice the former values.

Because the absolute values for Guatemalan cauliflower were at such variance from reported values,

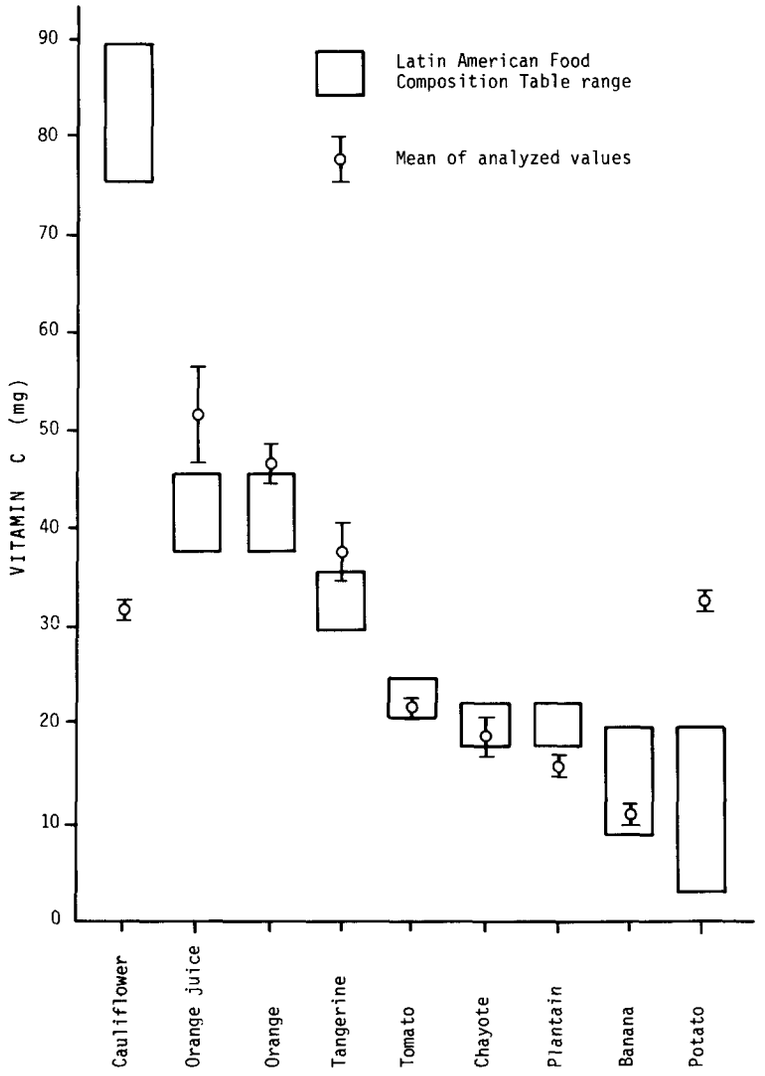

Fig. 2. Values for total vitamin C from the present study in relation to corresponding values in the Latin American food tables.

Table 3. Storage time and cooking affecting ascorbic acid and dehydroascorbic acid content in cauliflower

Food	L-AA (mg per 100 g)	L-DHAA (mg per 100 g)	Total vitamin C (mg per 100 g)
Cauliflower (USA)			
Raw	50·0	36·0	86·0
Cooked fresh	61·0	—	61·0
Cooked 1 week storage	55·0	—	55·0
Cauliflower (Guatemalan)[a]			
Cooked	27·3	4·7	32·0

[a] Arrived after shipping with mold.

analysis of a local Maryland variety was conducted (Table 3). The value for total vitamin C for the raw, North American cauliflower, 86 mg/100 g, was in exact agreement with the value in the Latin American table. The cooking of both freshly purchased and frozen-stored cauliflower resulted in about a 30% loss of total vitamin C activity; however, both local samples when cooked had over 40% greater amounts of the vitamin than the sample imported from Guatemala. Of interest is the fact that all cooked specimens of cauliflower, from Central or North America, had almost all of their vitamin C in the reduced form. The raw sample had 42% in the oxidized form.

DISCUSSION

The recommended daily dietary intakes of vitamin C for adult range from 30 mg (FAO, 1971) to 60 mg (National Research Council, 1980). An obvious epidemiological reality in Guatemala, as in most developing countries, is the absence of clinical scurvy as a public health problem; clearly, antiscorbutic levels of vitamin C occur in the typical diet of Guatemala. As the research branch of the National Committee for the Blind and Deaf of Guatemala, CeSSIAM was naturally interested in the emerging issue of vitamin C and cataractogenesis. A survey among 104 cataract patients revealed a median estimated vitamin C intake in the 7 days prior to hospitalization of 80 mg per day. We were able to describe the pattern of food intake that contributed to overall vitamin C intake. The eight food items chosen for analysis here were among the chief contributors for our population (Barrantes de González & Solomons, 1987).

Despite our predilection for the ophthalmological context, it would seem that all three of the aforementioned issues related to the oxidation state of the vitamers of vitamin C—iron absorption, nitrosamine formation and carcinogenesis, and cataractogenesis—are relevant to the Guatemalan population. Iron deficiency anemia was found to be common in the Republic during the Central American survey of 1965–67 (ICNND-INCAP, 1972). This most probably relates to poor *biological availability* of iron, rather than to an insufficient intake (Viteri *et al.*, 1972). Meat consumption is low among the poorer segments of the population of Guatemala, but when it is consumed, it is likely to have been grilled over a wood or charcoal fire, a prime condition for the formation of nitrosamines (Kim *et al.*, 1982). National and regional cancer incidence statistics do not exist in Guatemala, but it is the impression in gastroenterological circles that gastric cancer is not uncommon (Schneider, R. E., Personal communication). Finally, with the high altitude residence, and day-long outdoor agricultural pursuits in the highlands of Guatemala, sun exposure with low filtration of ultraviolet light is the plight of many. These are propitious conditions for the formation of cataracts (Zigman *et al.*, 1979; Taylor *et al.*, 1988). Again, incidence statistics are lacking, but in one hospital of the National Committee for the Blind and Deaf, 'Hospital Dr Rodolfo Robles V.', which is located in Guatemala City, a total of 978 cataract extractions were performed from January to December of 1984.

It was felt that a key component of the puzzle surrounding vitamin C and health in Guatemala would be food composition analysis, with specific emphasis on the partition among the chemically distinct vitamers. It was unavoidable that samples had to be frozen and shipped from Guatemala to the USA for the type of roboticized, HPLC analysis required. This had an obvious potential to affect the content and the composition of vitamin C in the foods. We were surprised by the high degree of correspondence between our current total vitamin C levels and those published over 25 years ago. The difference in the analytical methods, international transport of samples, and the cooking of those foods that are customarily consumed in that form, would all be expected to introduce variance with the earlier analyses. The absence of difference provides an argument against significant vitamin C depletion of our samples during freezing, storage, thawing and cooking. Whether the conditions of our study altered the partition of L-AA and L-DHAA within the total vitamin C pool is more difficult to judge. Bushway and colleagues conducted stability tests to see how long fruit and vegetable samples were stable in a solution of buffer and orthophosphoric acid. They demonstrated that vitamin C begins to degrade within 15 min after homogenization and that by 30 min 4–5% is oxidized. In order to determine the repeatability of the method, 22 fruits and vegetables were analyzed six times each for their vitamin C content and they concluded that the HPLC method for the determination of ascorbic acid in fruit, vegetables and juices was precise and that it was rapid and could be used for nutritional labeling and for the study of the effects of cultural and processing treatments on the vitamin C content of foods (Bushway *et al.*, 1988). Our one experiment with the local and Guatemalan frozen cauliflower—both of which had their vitamin C in a largely reduced form when sampled raw—suggests that freezing-thawing had no influence on chemical form. The same experiment would suggest, however, that cooking may, indeed, alter the chemical form of vitamin C. Specifically, the major reduction in the absolute vitamin C content of the Guatemalan cauliflower could be due to the discoloration and

fungal proliferation that was present at the time of preparation of the sample for analysis. In another study conducted also by Bushway and colleagues, it appears that the quality of freshness for eight different vegetables purchased at different markets, did not have a major impact on vitamin C concentrations, since only three of the fresh items were significantly higher in ascorbic acid content, so this tended to indicate that handling and storage were not influencing concentrations drastically (Bushway *et al.*, 1988). Alternatively, factors such as ripeness, genetic variety, temperature, sun-exposure, soil, fertilizer, and soil-nitrogen (Erdman & Klein, 1982) could all be responsible for the reduced vitamin C content.

We were able to establish that, indeed, there is a significant variance between common sources of vitamin C in the Guatemalan diet, not only in total content, but in the relative proportions of the reduced and oxidized forms. It would stand to reason, that on a weight for weight basis, the vitamin C from raw oranges or cooked chayote would have a greater potential to enhance the absorption of inorganic iron or reduce the burden of nitrosamines from charred meat, than would the vitamin C from tomatoes or banana. How the dietary form of the vitamer would influence the vitamin C portion in the crystalline lens is a matter for speculation. Human metabolism has the capacity to reduce and oxidize vitamin C within the body. Whether the eye might take up vitamin C in its first pass circulation following a meal, thus having a new supply that reflected the dietary forms, remains to be determined.

CONCLUSION

The automated, roboticized HPLC-based analytical procedure allows for the analysis of the specific vitamers of vitamin C. The values for total vitamin C in eight selected Guatemalan foods were, in general, very similar to those reported over 25 years ago in the Latin American Food Composition Tables, but in five of the foods, a mixture of both reduced and oxidized forms of the vitamin were found. Oranges and chayote had all of their vitamin C as L-AA and all of the vitamin in banana was the L-DHAA form. It is likely that the partition among vitamers of dietary vitamin C intake will influence the function of the vitamin as a promoter of iron absorption, as a protective factor against nitrosamine exposure, and even as a modifier of photooxidation in the human lens. More specific information on the vitamer forms of vitamin C in additional foods and in mixed diets will help us to understand epidemiological associations and to plan dietary interventions in high-risk groups.

ACKNOWLEDGEMENTS

We are indebted to Dr Joseph Vanderslice from the Nutrient Composition Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, USDA, Beltsville, Maryland for his collaboration in analyzing the food samples.

Appreciation is due to Dr Gary Beecher for his personal intervention in the international transport of the samples.

REFERENCES

Barrantes de González, L. & Solomons, N. W. (1987). Ingesta de vitamina C en pacientes con cataratas operados en el Hospital de Ojos y Oídos 'Dr Rodolfo Robles V.'. Presented at: I Scientific Week of the Faculty of Chemistry and Pharmacy of the University of San Carlos of Guatemala, Guatemala City, September, 1987.

Bensch, K. C., Fleming, J. E. & Lohmann, W. (1985). The role of ascorbic acid in senile cataract, *Proc. Nat. Acad. Sci.*, **82**, 7193–6.

Blondin, J., Baragi, V. K., Schwartz, E. R., Sadowski, J. & Taylor, A. (1986). Delay of UV-induced eye lens protein damage in guinea pigs by dietary ascorbate. *J. Free Rad. Biol. Med.*, **2**, 275–81.

Blondin, J. & Taylor, A. (1987). Measures of leucine aminopeptidase can be used to anticipate UV-induced age-related damage to lens proteins. *Mech. Aging Dev.*, **41**, 39–46.

Bushway, R. J., King, J. M., Perkins, B. & Krishnan, M. (1988). High-performance liquid chromatographic determination of ascorbic acid in fruits, vegetables and juices. *J. Liquid Chromatogr.*, **11**, 3415–23.

Bushway, R. J., Helper, P. R., King, J., Perkins, B. & Krishnan, M. (1989). Comparison of ascorbic acid content of super-market versus roadside stand produce. *J. Food Qual.*, **12**, 99–105.

Chandra, D. B., Varma, R., Ahmad, S. & Varma, S. D. (1986). Vitamin C in the human aqueous humor and cataracts. *Int. J. Vit. Nutr. Res.*, **56**, 165–8.

Erdman, J. W., Jr & Klein, B. P. (1982). Harvesting, processing and cooking influences on vitamin C in foods. In *Ascorbic Acid: Chemistry, Metabolism and Uses*, ed. P. A. Seib & B. M. Tolbert. American Chemical Society, Washington, DC, pp. 491–532.

FAO/WHO (1971). *Requirement of Ascorbic Acid, Vitamin D, Vitamin B12, Folate and Iron.* Technical Series No. 452, Rome, pp. 25–31.

Fox, F. W. & Levy, L. F. (1936). Experiments confirming the antiscorbutic activity of dehydroascorbic acid and a study of its storage and that of ascorbic acid by the guinea pig at different levels of intake. *Biochem. J.*, **30**, 211–17.

Hawk, G. L., Little, J. N. & Zenie, F. H. (1982). A robotic approach to automated sample preparation. *Int. Lab.*, **12**, 48–56.

Interdepartmental Committee of Nutrition for National Defense & Institute of Nutrition of Central America and Panama (1972). *Nutritional Evaluation of the Population of Central America and Panama, 1965–1967.* Regional Summary, DHEW Publication No. HSM-72-8120, Washington, DC.

Kim, Y-K., Tannenbaum, S. R. & Wishnok, J. S. (1982). Effects of ascorbic acid on the nitrosation of dialkyl amines. In *Ascorbic Acid: Chemistry, Metabolism and Uses*, ed. P. A. Seib & B. M. Tolbert. American Chemical Society, Washington, DC, pp. 571–85.

Monsen, E. R., Hallberg, L. & Layrisse, M. *et al.* (1978). Estimation of available dietary iron, *Am. J. Clin. Nutr.*, **31**, 134–41.

National Research Council—Food and Nutrition Board (1980). *Recommended Dietary Allowances*, 9th rev. ed. National Academy of Sciences, Washington, DC, 185 pp.

Pirie, A. & Van Heyningen, R. (1956). In *Biochemistry of the Eye.* Charles C. Thomas, Springfield, IL, p. 24.

Roe, J. H. & Barnum, G. L. (1936). The antiscorbutic potency of reversibly oxidized ascorbic acid and the observation of an enzyme in blood which reduces the reversibly oxidized vitamin. *J. Nutr.*, **11**, 359–69.

Russell, P., Garland, D., Zigler, J. S. Jr, Meakin, S. O., Tsui, L-C. & Breitman, M. L. (1987). Aging effects of vitamin C on a human lens protein produced in vitro. *FASEB J.*, **1**, 32–5.

Taylor, H. R., West, S. K., Rosenthal, F. S., Munoz, B., Newland, H. S., Abbey, H. & Emmett, E. A. (1988). Effect of ultraviolet radiation on cataract formation. *N. Engl. J. Med.*, **19**, 1429–33.

Tuderman, L., Myllyla, R. & Kivirikko, K. I. (1977). Mechanism of the prolyl hydroxylase reaction. 1. Role of co-substrates. *Eur. J. Biochem.*, **80**, 341–8.

Vanderslice, J. T. & Higgs, D. J. (1985). Robotic extraction of Vitamin C from food samples, *J. Micronut. Anal.*, **1**, 143–54.

Varma, S. D., Chand, D., Sharma, Y. R., Kuck, J. F., Jr & Richards, R. D. (1984). Oxidative stress on lens and cataract formation: role of light and oxygen. *Curr. Eye Res.*, **3**, 35–57.

Viteri, F. E., de Tuna, V. & Guzmán, M. A. (1972). Normal haematological values in the Central American population. *Brit. J. Haematol.*, **23**, 184–204.

Wu-Lueng, W-T. & Flores, M. (1961). *Tabla de Composición de Alimentos para Uso en América Latina.* Interdepartmental Committee of Nutrition of the National Defense, National Institute for Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland, USA and Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala, 132 pp.

Zigman, S., Datiles, M. & Torozynski, E. (1979). Sunlight and human cataracts. *Invest. Ophthalmol. Vis. Sci.*, **18**, 462–7.