SPECTROPHOTOMETRY DETERMINATION OF IRRADIATED ASCORBIC ACID

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ABSTRACT

This work aims at the study of the radiation effects on vitamin C, when irradiated either solid, as a powder, or in an acetic-metaphosforic acid solution, irradiated with Co-60 or electron beam irradiation (EB). Doses of Co-60 γ radiation of 0, 0.05, 0.1, 0.25, 0.5, 1.0, 5.0, 10, 20 and 50kGy were used for both solid or liquid samples. EB irradiations with doses of 0, 5, 10, 20, 50, 100, 200 and 300kGy were only employed for solid state samples. For samples irradiated in solution, a decrease of the UV absorbance as a linear function of dose was shown for doses of 0.05, 0.1, 0.25 and 0.5kGy remaining constant afterwards. UV spectra of samples irradiated in the solid state and dissolved after irradiation did not change as a function of dose showing no effect of γ or EB irradiation on ascorbic acid in this case. These results point out the importance of the water content on vitamin C stability and suggest several practical considerations that can be drawn on industrial food irradiation.

I. INTRODUCTION

Although associated especially with oranges and lemons, ascorbic acid (AA) or vitamin C is found in varying amounts in almost every kind of food except sugar, confectionery, dried cereals and pulses. The most important sources of vitamin C in human nutrition, however, are fresh, frozen or canned fruits and fruit juices, vegetables and potatoes. Unlikely to most of the other vitamins, vitamin C is required by only a few animal species, human beings among them[1].

Irradiation of fresh plant products is generally limited to low-dose applications, since higher doses harm these foodstuffs. Desirable results at doses up to 1kGy include delay of ripening of some fruits and insect disinfestation. Vitamin C is commonly considered as a radiation-sensitive vitamin. However, many factors influence the radiation resistance of a vitamin, such as the composition of the food under consideration, the packaging atmosphere and the temperature during irradiation and postirradiation storage. For instance, no loss of this vitamin was found in ground paprika irradiated at sterilizing dose[2] or onion powder even when a extremely high dose of 270kGy was applied[3].

When foods are exposed to ionizing radiation under conditions envisioned for commercial application, the effects upon nutrients are not markedly different in degree from those observed with other food preservation methods. Because of the protective qualities inherent in foods, the sensitivity of nutritional components is less than that of the same nutrients irradiated in pure form or in artificial solutions and mixtures. Thomas and Calhoun[4] discussed nutritional aspects of food irradiation, among them vitamin C activity retention.

Irradiation as a preservation method is replacing the ethylene dibromide treatment that is going to be banned worldwide in a near future[5]. Moy[6] compared irradiation and thermal treatments and concluded that irradiation, especially gamma radiation, showed to be superior to thermal methods for papayas and other tropical fruits in efficacy, product quality and economics as a quarantine treatment.

A variety of analytical procedures exists for the detection of ascorbic acid. Nevertheless, no procedure is entirely satisfactory due to the lack of specificity and the number of interfering substances contained in most foodstuffs[7]. This work aims at the study by spectrometry of radiation effects on pure vitamin C, when irradiated

either solid, as a powder, or in an acid solution, irradiated with Co-60 or electron beam irradiation (EB).

II. MATERIAL AND METHODS

All reagents used were of analytical grade and distilled water was used throughout the work. A 8% acetic acid solution and a 3% (w/v) metaphosphoric acid - 8% (v/v) acetic acid solution was assayed. Media were kept in a refrigerator and used for preparing the dilute solutions.

An ascorbic acid stock solution (100μg.ml^{-1}) was freshly prepared and for the calibration curve an standard solution of 10μg ml^{-1} was used (2.5 - 10.0 μg.ml^{-1}).

A ^{60}Co Gammacell 220 from Atomic Energy of Canada Ltd, with 471Ci activity and dose rate of 0.338 kGy.h^{-1} was employed.

A Dynamitron (Radiation Dynamics RDI) electron beam (EB) accelerator, 0.634 MeV, 1.7 mA, scan rate of 3.36 m.min^{-1} (5 kGy each time) was used.

An INTRALAB-DMS 100 UV/Vis spectrophotometer was used from 200 to 400 nm, having a path length of 1 cm, volume of 3 ml. Maximum absorbance values from each spectrum (from 241.4 to 245.4 nm) were used for intercomparison, instead of a fixed wavelength for all the samples.

Three experiments were performed:

Sγ Samples. The ascorbic acid as a powder was initially γ-irradiated at 0.5, 1.0, 5.0, 10, 20 and 50kGy and then dissolved, diluted and the absorbance of the solution read in the spectrophotometer.

Lγ Samples. The ascorbic acid was dissolved (10μg.ml^{-1}) and aliquots were γ-irradiated with 0, 0.05, 0.1, 0.25, 0.5, 1.0, 5.0, 10, 20 and 50kGy and the absorbance measured.

EB Samples. Solid ascorbic acid was initially irradiated with electrons with doses of 0, 5, 10, 20, 50, 100, 200 and 300kGy and then dissolved, diluted and the absorbance measured.

Calibration Curve. Dilutions of the AA standard solution: 2.5, 5.0, 7.5, and 10 μg.ml^{-1} was used to plot absorbance vs concentration at 243.6 nm.

III. RESULTS AND DISCUSSION

In order to choose the best medium for study radiation effects on vitamin C, the stability of the two solutions to be used as solvents was studied. Figure 1 shows the absorption spectra of 100μg.ml^{-1} ascorbic acid in 8% acetic acid and their stability upon time. As it can be seen, the solution is unstable, having on the 10th day only 53% of the initial absorbance, and the 15th, only 13%. Table 1 shows the corresponding data as regards to the stability of the 10μg.ml^{-1} ascorbic acid in 3% methaphosphoric-8% acetic acid solution. The spectrophotometric readings up to 10 days after preparation are presented in this table. As it can be seen, no apparent differences were observed. Though, the second solvent was found to be the most suitable, as it remained stable as long as 10 days.

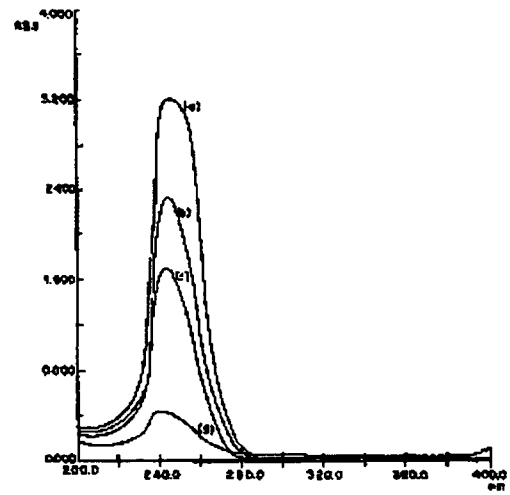


Figure 1. Absorption spectra (100μg.ml^{-1}.in medium 8% acetic acid) measured after (a) one day, (b) eight days, (c) ten days, (d) fifteen days

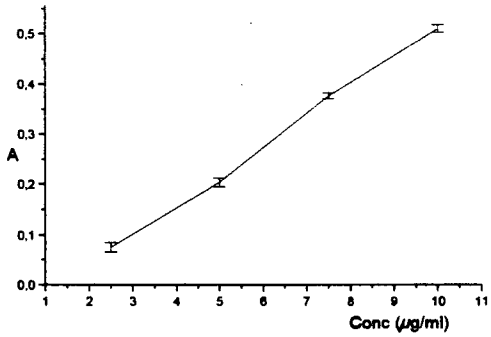
TABLE 1. UV-Absorbance values for 10μg.ml^{-1} ascorbic acid solution (medium: 3% methaphosphoric- 8% acetic acid)

Days (after prepared)	Absorbance
01	0.537
01	0.511
03	0.535
03	0.552
03	0.519
08	0.527
10	0.519

Spectrophotometric readings from Sγ and EB samples irradiated respectively with gamma and electrons radiations are shown in Table 2. No apparent differences appeared with the increase of the radiation dose, neither for gamma nor EB irradiation were found when the vitamin C samples were irradiated in the solid state, even when irradiated with doses as high as 300kGy.

TABLE 2. Absorbance values for Sγ and EB Series

Sample (Doses in kGy)	Absorbance
Sγ-0.5	0.512
Sγ-1.0	0.534
Sγ-5.0	0.500
Sγ-10	0.494
Sγ-20	0.623
Sγ-50	0.600
EB-5	0.500
EB-10	0.496
EB-20	0.480
EB-50	0.530
EB-100	0.601
EB-200	0.576
EB-300	0.561



y = 0.0591x - 0.0768 (r = 0.9983)

Figure 3. Calibration Curve: Absorbance at 243.6nm vs Concentration of Ascorbic Acid Standard Solutions

The average of duplicate spectrophotometric readings from Lγ samples (vitamin C irradiated in the aqueous solution) are presented in Table 3 as a function of dose. As it can be observed, the absorbance decreased considerably until 0.5 kGy, remaining almost constant afterwards. For doses from 0 to 0.25 kGy the points can be ajusted to a linear function:

$$y = -1.7293x + 0.5177 \qquad (r = 0.9923)$$

TABLE 3. Doses, Absorbance Readings and Standard Deviation (Lγ samples)

DOSE (kGy)	A	SD
0	0.544	0.012
0.05	0.418	0.025
0.1	0.320	0.004
0.25	0.098	0.001
0.5	0.051	0.000
1	0.052	-
5	0.076	0.005
10	0.063	0.004
20	0.098	0.006

In a liquid system, even in an stable medium like the one employed, the vitamin C stability against radiation was not preserved. The retention of vitamin C activity measured by spectrophotometry was 18% for a dosis of 0.25 kGy.

The spectrophotometric values for ascorbic acid standard solutions (2.5 - 10.0 $\mu g.ml^{-1}$) were measured at 243.6 nm. Linear relationship was obtained between absorbance and ascorbic acid concentration as presented in Figure 3.

In this work, a UV-spectrophotometric method was used directly for the determination of ascorbic acid. In this case, this was possible because the solutions contain pure ascorbic acid and there were no interferences. For a further development a third order derivative spectrophotometer was described for the determination of vitamin C. Özgür and Sungur[8] utilized this method to determine ascorbic acid in parsley, grapefruit and kiwi, eliminating matrix effect by derivatization of the absorption spectra.

Other authors[9] described separation and determination of soluble vitamins by means of reversed-phase high performance liquid chromatographic method (HPLC) with ultraviolet spectrometrical detection too.

The main sources of vitamin C in the Western diet are fruits and vegetables, including potatoes. Total vitamin C activity is the sum of ascorbic acid (AA) and dehydroascorbic acid (DHAA) actitity[10]. Freshly harvested produce contains mostly AA and many workers only determine AA as a measure of vitamin C. In fact this was the case in the present work where only ascorbic acid was determined.

In this case it was shown that vitamin C supports extreme high doses of radiation in the absence of water. Meanwhile, when pure ascorbic acid is irradiated in an aqueous solution there are a loss of activity in the dose range used for fruit irradiation disinfestation, i.e., up to 1 kGy.

Although ascorbic acid destruction under ordinary conditions is chiefly due to oxidation, it is not autoxidizable within the normal pH range of plant and animal tissues[11]. In fact the rate of its oxidative destruction depends both upon its oxidation-reduction potential and a delicate interdependence of pH and the concentrations of other factors and components of the food.

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