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Influence of concentration on the radiolytic decomposition of thiamine, riboflavin, and pyridoxine in aqueous solution

Abstract

Vitamin loss during irradiation has been claimed as a critical area in food irradiation technology, especially that of thiamine (B1), which has been considered as the most sensitive to radiation. Although it has been suggested that no vitamin deficiency could result from consuming irradiated food, a long debate on the loss of vitamins and other nutrients during food irradiation has been maintained by the lack of experimental studies monitoring decomposition rates at different concentrations and doses. Since thiamine, riboflavin, and pyridoxine are labile vitamins, this study has focused on their radiolytic decomposition in dilute aqueous solutions in the presence of air. The decomposition process was followed by HPLC and UV-spectroscopy. The results obtained in aqueous solutions showed a dependence of the decomposition as a nonlinear function of the dose. Of these three compounds, the decomposition was higher for thiamine than for riboflavin and even less in pyridoxine.

Key words: thiamine, riboflavin, pyridoxine, vitamins, radiolysis, decomposition.

Influencia de la concentración en la descomposición radiolítica de tiamina, riboflavina y piridoxina en solución acuosa

Resumen

La pérdida de vitaminas durante procesos de irradiación ha sido considerada como un área crítica en la tecnología de irradiación de alimentos, especialmente la tiamina (B1), que ha sido considerada como la más sensible a la radiación ionizante. La deficiencia de vitaminas en humanos no es producida por el consumo de alimentos irradiados, sin embargo, existen debates sobre la pérdida de vitaminas y otros nutrientes provocada por la irradiación de alimentos, esta discusión sigue latente debido a que hay pocos estudios experimentales de la descomposición de vitaminas a diferentes dosis y concentraciones. Esta investigación se centró en el estudio de la descomposición radiolítica de tiamina, riboflavina y piridoxina en soluciones acuosas y en presencia de aire. El proceso de descomposición fue seguido por cromatografía líquida con detección UV. Los resultados obtenidos en soluciones acuosas mostraron una dependencia no lineal entre la descomposición en función de la dosis. De estos tres compuestos, la descomposición fue mayor en tiamina que en riboflavina y menor en la piridoxina.

Palabras clave: Tiamina, riboflavina, piridoxina, vitaminas, radiólisis, descomposición.

Influência da concentração na decomposição radiolítica de tiamina, riboflavina e piridoxina em solução aquosa

Resumo

A perda de vitaminas durante processos de irradiação tem sido considerada uma área crítica na tecnologia de irradiação de alimentos, especialmente no caso da tiamina (B1), que tem sido considerada como a mais sensível à radiação ionizante. Embora a deficiência de vitaminas em seres humanos não seja produzida pelo consumo de alimentos irradiados, longos debates sobre as perdas de vitaminas e outros nutrientes causadas pela irradiação de alimentos tem sido mantidos devido aos estudos experimentais limitados monitorando a proporção da decomposição em diferentes concentrações de vitaminas e doses de radiação aplicadas. Considerando que a tiamina, riboflavina e piridoxina são vitaminas instáveis, o presente estudo focalizou a decomposição radiolítica dessas vitaminas em soluções aquosas diluídas e na presença de ar. O processo de decomposição foi analizado por cromatografia líquida com detecção UV. Os resultados obtidos em soluções aquosas mostraram uma dependência da decomposição como função não linear da dose. Destes três compostos, a descomposição foi mais alta para tiamina que na riboflavina e menor para piridoxina.

Palavras-chave: tiamina, riboflavina, piridoxina, vitamina, radiolisis, decomposição.

Introduction

Food irradiation is a physical method applied for preservation and storage of a foodstuff (1) and, among the many advantages, this process reduces pathogen microbes while preserving nutritional value. Certainly, the most important benefit of this technology from the microbiological point of view is the high quality of irradiated food because, depending on the dose, the process destroys common disease-causing microorganisms, among which are *Salmonella spp*., *E. coli*, *Clostridium botulinum*, etc. (2). The interest in food irradiation is high in order to avoid economic losses worldwide, caused by contamination and degradation during their transportation from the production to the consumption centers. This treatment applies to fruits, vegetables, red meat, chicken, fish, and seafood. Shelf-life can be considerably extended with a combined treatment of low-dose irradiation and cooling, without altering taste or texture (3). Nonetheless, some disadvantages of food irradiation include the breakdown of vitamins.

Vitamins are organic compounds that are easily affected during the processes of conservation, in particular under treatments applying the irradiation doses commonly used for food preservation (1, 3-6). Vitamins are essential micronutrients for the metabolism of living beings since they are necessary for growth and proper bodily function of organisms (7). Therefore the mechanisms of vitamin loss caused by the action of ionizing radiation are of vital importance in radiation chemistry studies as are the parameters that modify this destruction, mainly in aqueous solution (8-15). On the other hand, it should be remembered that radiation does not behave in the same or similar ways in all types of products and the degree of vitamin destruction depends, among other variables, on the composition of the food and on its water content, on the irradiation dose, and on the nature and the concentrations of the vitamins (3, 9, 10, 16-18).

In order to clarify the radiolytic process of vitamin loss during irradiation this paper has examined different important vitamins frequently destroyed during food preservation, such as thiamine, riboflavin, and pyridoxine. The reason to study the effects of gamma irradiation on these vitamins relates to the fact that food contains a high percentage of water and these vitamins are easily dissolved on it. The aim of this work is to provide an experimental framework for comparison during conventional food irradiation.

Materials And Methods

Materials

The irradiation vials were cleaned with a sulfuric-nitric acid mixture, rinsed with plenty of running water, then washed in bidistilled water and deionized water, and subsequently dried at 250 °C for two hours. The reagents used were of the highest purity existing on the market and were used without further purification. Thiamine, riboflavin, and pyridoxine, in addition to 1-hexane-sulphonic acid (sodium salt), acetic acid, and methanol were from Aldrich. The water used was obtained from a compact Milli-Q Plus Water System.

Samples

Solutions of vitamins were prepared in concentrations ranging from 0.25 to 2 mmol/L for thiamine and pyridoxine, and from 0.05 to 0.25 mmol/L for riboflavin.

Irradiation

Solutions for each of the substances were prepared by progressive dilution of stock solutions prepared by carefully weighing appropriate amounts of each one of the solids. Aliquots (3 mL) of each vitamin solution were irradiated using glass cells closed with Thermogreen LB-1 plugs. Samples were irradiated to different doses with a cobalt-60 gamma source (Gamma-beam 651 PT, MDS Nordion, containing 1.85 x 10^{12} Bq of Co-60) using a dose rate of 13.74 Gy/min at 22 °C, calibrated with Frick dosimetry (19).

Liquid chromatography

Analyses were carried out immediately after irradiation by high performance liquid chromatography. These analyses were performed with an Agilent Technologies, LC series 1100 HPLC (degasser, quaternary pump, autosampler, and diode array detector). The separations were carried out isocratically on a 150 mm x 4.6 mm i. d. Hypersil ODS 5 µm column. The optimized mobile phase was a mixture of 80 % *v/v* aqueous 5 mmol/L 1-hexanesulfonic acid (sodium salt) solution containing 1 % *v/v* glacial acetic acid and 20 % *v/v* methanol at a flow rate of 0.4 mL/min. The volume injected was 100 µL. The detector was used to record spectral data from 200 to 400 nm in a 3-D format. Appropriate spectra and chromatograms were extracted from these recordings. Concentrations of the vitamins were determined from peak areas at appropriate wavelengths.

Results

The results of decomposition of vitamins due to the absorbed dose are an average of three or more irradiated samples, which were analyzed by chromatography. Possible errors are largely limited by system behavior and are estimated to correspond to a standard deviation of less than 3 %.

Effect of irradiation dose on thiamine (vitamin B1) in aqueous solution

Gamma irradiation produced significant changes in the aqueous solution of thiamine (Vitamin B1), as indicated in Figure 1A, which shows the chromatographs of a 1 mmol/L aqueous solution of thiamine irradiated to increasing doses of 0, 0.35, 0.7, 1.05, 1.40, 1.75, and 2.10 kGy.

The effects of the irradiation dose for thiamine can be observed mainly in three peaks (or domains). Peak 1 contains several radiolytic products including pyrimidine and thiazole, formed during the irradiation process in quite small quantities. The area under peak 3 shows that, as the absorbed dose increased, the thiamine concentration decreased accordingly.

This result also shows a new compound (peak 2) emerging as thiamine decomposition continues, increasing as the dose increases. Although this main radiolytic product (peak 2) has not been identified, this product presented a different UV spectrum from either of the two rings which form the vitamin, 4-methyl-5-(β-hydroxyethyl)-thiazole and 2,5-dimethyl-4-amino-pyrimidine (Figure 1B).

As shown in Figure 1B, some radiolytic products were formed during thiamine irradiation. Products 1 and 2 absorb at different wavelengths than thiamine, and also do not correspond to thiazole (t_R = 5.5 min). This result indicates that thiamine did not simply break into its two rings (20).

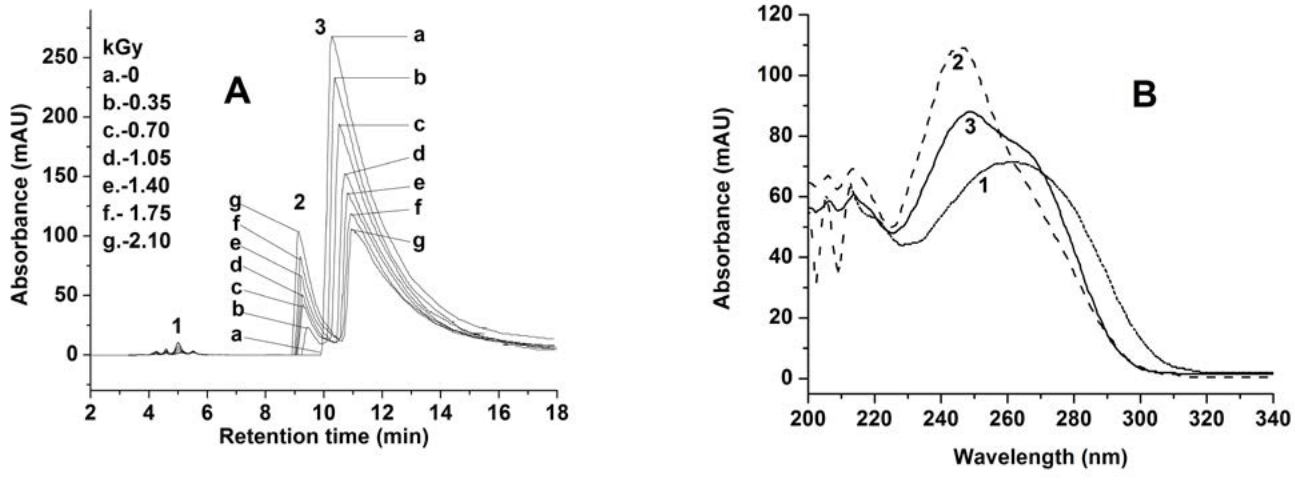

Figure 1. A. Representative chromatograms from irradiated samples of aqueous solutions of thiamine, recorded at 245 nm. Irradiation doses were 0, 0.35, 0.70, 1.05, 1.40, 1.75, and 2.10 kGy. Peak identification: 1) secondary radiolytic products (including the pyrimidine and thiazole); 2) main radiolytic product; 3) thiamine. **B.** UV spectra of thiamine and its radiolytic products: 1) secondary radiolytic product, $t_R = 5$ min; 2) main radiolytic product, $t_R = 9.3$ min; 3) thiamine.

As shown in Figure 2, the decomposition of thiamine in aqueous solution due to irradiation occurs even at low doses. Since the radiation effects depend on dose variation, it was very important to monitor structural changes in thiamine. Table 1 shows the percent loss of thiamine (1 mmol/L) at different doses administered at a dose rate of 13.74 Gy/min.

Doses of 0.1 to 1 kGy are used to inhibit infestations (21). For example, the dose used for the disinfection of grains is about 0.6 kGy. In contrast, an irradiated aqueous solution of 1 mmol/L of thiamine lost about 25% of the target compound. These results are also consistent with other studies (9, 17-18) that indicate that higher irradiation doses, similar to those employed to eliminate pathogenic micro-organisms such as *Salmonella* (2.5–3.0 kGy) induce decomposition of the vitamin higher than 50%.

Effects of irradiation dose on pyridoxine (vitamin B6) in aqueous solution

Figure 3 shows the chromatograms of a 0.25 mmol/L solution of pyridoxine irradiated with different doses. The peak of pyridoxine decreases as the dose increases; at the same time, new peaks are detected whose areas increase as the dose increases. The radiolytic products formed have been identified, as indicated in Figure 3, in an earlier paper by Albarran *et al.*, 2008 (15). In that paper the radiolytic products were analyzed by HPLC equipped with photodiode array and mass detectors.

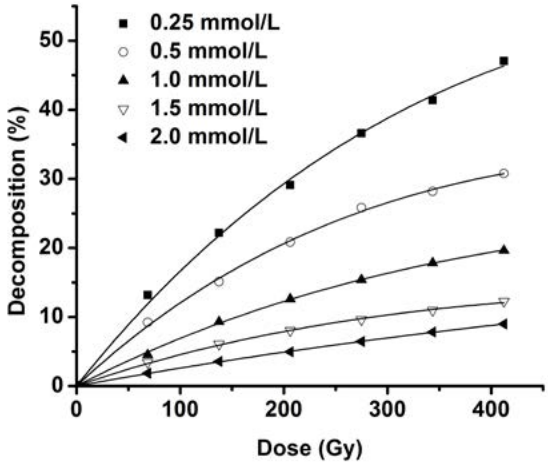

Figure 2. Decomposition (%) of thiamine in aqueous solution as a function of dose. Thiamine concentrations varied from 0.25 mmol/L to 2 mmol/L and irradiation dose varied from 0 to 412 Gy.

The different decompositions percentages with respect to irradiation dose, with concentrations of the vitamin from 0.25 to 2.0 mmol/L, can be seen in Figure 4. The Table 1 shows the percent loss of pyridoxine (1 mmol/L) at different doses administered at a dose rate of 13.74 Gy/min.

The decomposition of pyridoxine in aqueous solution is less than that of the other two vitamins. This vitamin usually occurs in the form

Table 1. Percent loss of thiamine and pyridoxine at different radiation doses in aqueous solution (initial concentrations: 1 mmol/L).

Dose (kGy)	Loss of thiamine (%)	Loss of pyridoxine (%)
0[a]	0[a]	0[a]
0.07	4.5	2.5
0.14	9	4
0.20	12	—-[b]
0.25	13.8	7
0.27	15	8
0.34	17	9
0.41	20	11
0.50	23	13
1.00	33	23
1.25	38	—-[b]
1.50	42	33
1.75	46	37
2.00	50	40
2.25	53	—-[b]
2.50	57	—-[b]
2.75	59	—-[b]
3.00	61	—-[b]

[a] Unirradiated vitamins, 0 % loss of vitamin.
[b] Pyridoxine non-irradiated at these doses.

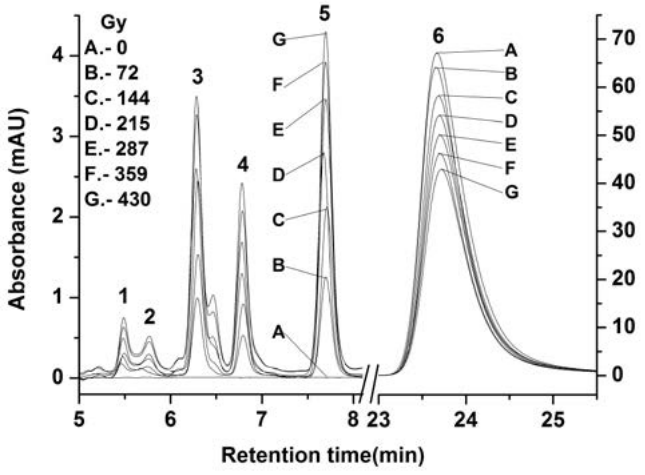

Figure 3. Chromatograms of 0.25 mmol/L pyridoxine irradiated to different doses, recorded at 234 nm. The radiolytic products are: 1) pyridoxy-3,6-quinone, 2) 2,4,5-trihydroxymethyl-3-pyridinol, 3) pyridoxal, 4) isopyridoxal, 5) 6-hydroxypyridoxine, 6) pyridoxine.

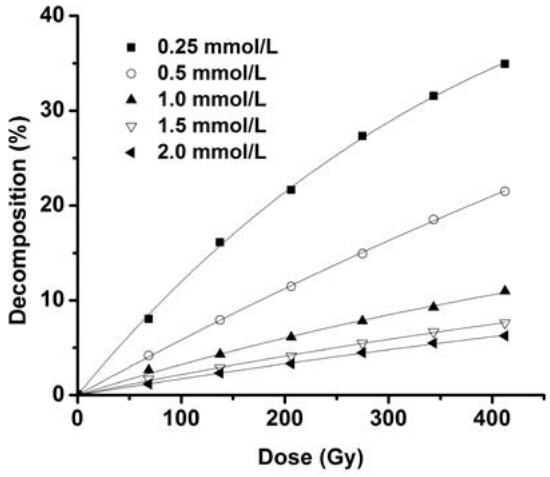

Figure 4. Decomposition (%) of pyridoxine in aqueous solution as a function of dose. Vitamin concentrations varied from 0.25 mmol/L to 2.0 mmol/L and irradiation doses varied from 0 to 412 Gy.

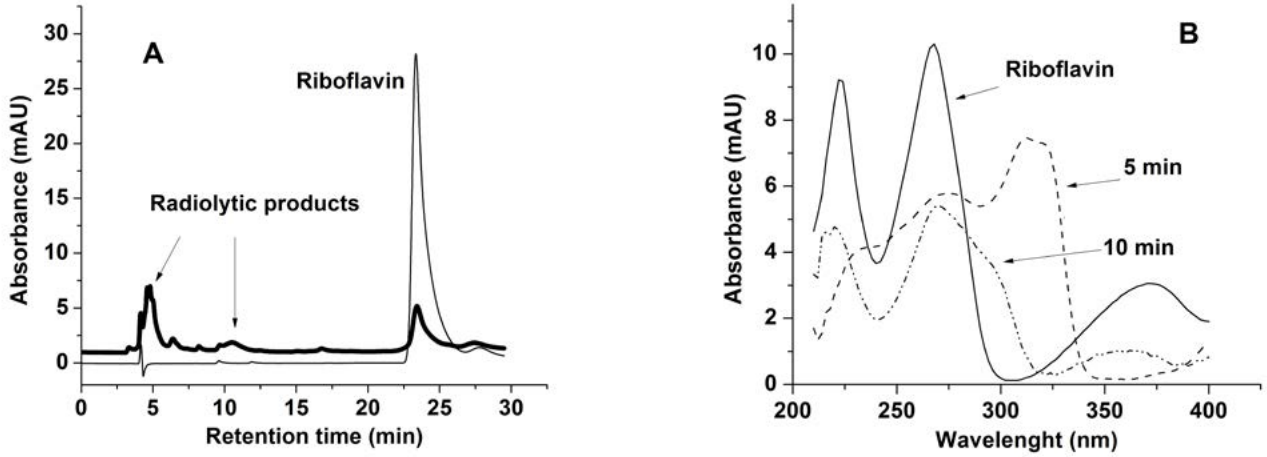

Figure 5. A. Chromatogram of a 0.05 mmol/L of riboflavin without irradiation (**––**) and irradiated at 2.1 kGy (––). **B.** UV spectra of radiolytic products (t_R = 5 and 10 min) and riboflavin (t_R = 23 min).

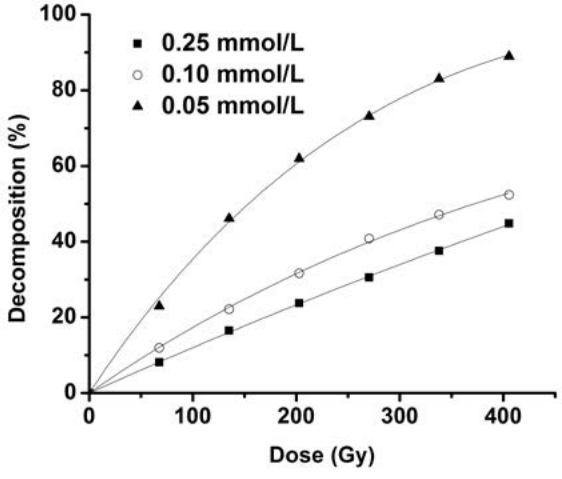

Figure 6. Decomposition (%) of riboflavin in aqueous solution at different concentrations as a function of dose.

Table 2. Loss of riboflavin to different radiation dose in aqueous solution. (Initial concentration 0.1 mmol/L)

Dose (kGy)	Loss of riboflavin (%)
0[a]	0[a]
0.35	31
0.70	951
1.05	1265
1.40	75
1.75	84
2.10	88

[a] Unirradiated vitamins.

of a coenzyme in metabolic processes. This may be why its radiolysis is somehow protected in food.

Effect of irradiation dose on riboflavin (vitamin B2) in aqueous solution

Figure 5A presents the chromatogram of a 0.05 mmol/L solution of riboflavin in aqueous solution (a) without irradiation and (b) subjected to a dose of gamma radiation of 2.1 kGy. This chromatogram shows a significant decrease of the riboflavin peak, as well as the emergence of other peaks with different retention times, representing the radiolytic products obtained after irradiation, although these products appeared in negligible concentrations at lower doses. Figure 5B shows the UV spectra of the two radiolytic products obtained after a dose of 2.1 kGy, which absorb at different wavelengths in comparison with the spectrum of pure riboflavin. These radiolytic products may be parts of riboflavin, however, they have not yet been identified.

A critical dose is observed for riboflavin, as deduced from Figure 6, which shows the decomposition percentage of aqueous solutions of riboflavin exposed to gamma radiation at different doses and varying concentrations (0.25, 0.1, and 0.05 mmol/L). The Table 2 shows the percent loss of riboflavin at different doses administered, (concentration 0.1 mmol/L). Due to the low solubility of the riboflavin, the results of the loss of this vitamin were not disclosed in the Table 1.

Because the maximum solubility of riboflavin is 0.01 mg in 100 mL of water (a concentration of 0.265 mmol/L), the highest concentration used in this experiment was 0.25 mmol/L. However, for practical purposes, this concentration is already too high since the concentration of this vitamin in food is less than 0.01 mmol/L. As an example, milk contains 0.17 mg in 100 mL, corresponding to 4.5 μmol/L in aqueous solution (22). Because the maximum solubility of riboflavin is 70 mg in 100 mL of water (a concentration of 0.18 mmol/L) (23), the highest concentration used in this experiment was 0.25 mmol/L. However, for practical purposes, this concentration is already too high since the concentration of this vitamin in food is less. As an example, milk contains 1.78 mg in 100 mL, corresponding to 0.047 mmol/L in aqueous solution (7).

On the other hand, the solution with the lowest concentration of riboflavin (0.05 mmol/L) shows a higher decomposition rate and the graph presents curvature, which is an indication of the formation of some secondary radiolytic products. This fact indicates that the riboflavin concentration decreased with increasing radiation dose and that the presence of radicals derived from water react with the primary radiolytic products. The other solutions present less curvature.

As presented in Figure 6 the rate of riboflavin decomposition is less than that of thiamine at the same dose of irradiation. Moreover, the riboflavin solution has a concentration ten times lower (0.1 mmol/L) than that used with the solution of thiamine (1 mmol/L), therefore its decomposition is readily noticeable. It should be pointed out that a significant loss of riboflavin occurs due to its high reactivity to light (23) and to interaction with radicals from the water produced during the irradiation process (11).

Discussion

This work describes the decomposition of thiamine, riboflavin, and pyridoxine in dilute aqueous solutions under low-dose gamma irradiation. All the vitamins exhibit radiation decomposition at low doses with the subsequent formation of new radiolytic products.

Because thiamine, riboflavin, and pyridoxine were irradiated in aqueous solutions at low concentrations, it is considered that radiation interacts with the water of the system producing fairly reactive species that then react with the vitamins, causing their decomposition. The decomposition of the vitamins is not proportional to the absorbed dose because the initial concentration of vitamins decreases as function of the dose, and the newly formed radiolytic products also compete for the free radicals, that is the oxidizing species $^{\bullet}OH$, $HO_2^{\bullet}$, and H_2O_2 (11) produced in the radiolytic decomposition of water.

Given the understandable requirement to use minimal radiation doses for food irradiation, this study was carried out under specific conditions such as low concentrations in aqueous solution and in the presence of oxygen. Since these experiments were different from the actual conditions of vitamin-containing foods, some irradiation effects could be modified by the presence of other sheltering biomolecules. Vitamins are often linked to enzymes or other molecules that protect them from ionizing radiation, diminishing the degree of decomposition.

Future studies should continue to examine the differential preservation effects of gamma irradiation on vitamins contained in food in order to explain and quantify differential decomposition of vitamins in food preservation or to design free radical scavengers for food protection during gamma irradiation of food.

Most importantly these studies on vitamin irradiation should help to overcome public resistance to irradiated products, sustained generally by the lack of information and fear of radiation, and will encourage further experimental studies on vitamins derived from different food products. Public awareness of the benefits of this technology in minimizing nutrient loss and maximizing product safety in our modern society remains an important goal.

Conclusions

The results obtained in this study indicate that there is a nonlinear dependence of the decomposition as a function of the dose; nevertheless, the decomposition seems to be strongly dependent on the concentration of the vitamins since at higher concentrations, less vitamin is lost. Comparing the three vitamins analyzed at the same dose (412 Gy) and concentration (0.25 mmol/L) it was deduced that the order of decomposition is thiamine > riboflavin > pyridoxine (47, 44, and 35 % respectively). The decomposition of vitamins takes place mainly because radicals from the radiolysis of water (^{-}OH, ^{-}H and hydrated electrons) attack them, mainly forming oxidized radiolytic products.

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