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DEGRADATION OF BIOGENIC AMINES BY GAMMA RADIATION PROCESS AND IDENTIFICATION BY GC/MS

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ABSTRACT

Biogenic amines are low molecular weight organic bases with aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) structures that can be found in several foods, in which they are mainly produced by microbial decarboxylation of amino acids. The reasons to control amines in food are their potential toxicity and their use like food quality markers. The consumption of food containing large amounts of biogenic amines can result in allergic reactions, characterized by difficulty in breathing, rash, vomiting, and hypertension. Biogenic amines are also known as possible precursors of carcinogens, such as N-nitrosamines. Traditionally, biogenic amine formation in food has been prevented, primarily by limiting microbial growth. However, control measures to reduce their levels once formed need to be also considered. The biogenic amines are frequently found in high concentrations and not reduced by high-temperature treatment, which makes difficult to use conventional methods of food preservation for this purpose. Food irradiation has been used in many countries for inhibition of sprouting, destruction of food borne insects, extension of shelf life or improvement of the technological of food. Irradiation is also known as a good method for inactivating pathogens and reducing microorganisms in food materials. Furthermore, besides the sanitary purpose, irradiation technology in new trials can be applied to induce radiolysis of toxic contaminants in food products reducing their content. This study has the objective to evaluate the effect of different gamma irradiation doses (1, 3 and 5kGy) in methanol solutions of three different biogenic amines: tryptamine, tyramine and b-phenylethylamine. The solutions were prepared using standard biogenic amines purchased from Sigma-Aldrich Brasil and methanol HPLC grade with a concentration of 100µg/mL. They were irradiated in Centro Tecnológico do Exército, using a research radiator with Cs-137 source and maximum dose rate of 1,8kGy/h. Dosimetry was performed with the calculation of the source exposure time using a specific program for the irradiator. For identification of amines it was used gas chromatography associated with a mass detector. The chromatographic conditions used were: interface temperature at 200°C, SCAN mode for m/z 15 to 300, helium mobile phase, manual injection, split rate of 1/5 and flow rate of 1.00mL/min. The heating was linear from 40°C to 300°C, with a rate of 10°C/min. Chromatographic separation was performed with column RTx-5MS, 30m x 0,25mm x 0,25µm, stationary phase 5% diphenyl - 95% dimethylpolysiloxane. The content of the amines after the irradiation process suffered considerable reduction. Tryptamine was more radiosensitive being reduced by about 85% at 1kGy, 99% at 3kGy and 100% at 5kGy. The b-phenylethylamine was reduced from its initial amount in about 20% at 1kGy, 70% at 3kGy and 85% at 5kGy. Tyramine showed less sensitivity to gamma radiation with reduction only 20% at 1kGy, 50% at 3kGy and 60% at 5kGy. These results show that the use of the irradiation process was efficient in the degradation of the amines tested.

1. INTRODUCTION

Amines are basic nitrogenous compounds in which one, two or three atoms of hydrogen in ammonia are replaced by alkyl or aryl groups [1]. When these amines are formed or degraded as part of the normal metabolism of microorganisms, plants and animals, in which they have important physiological functions they are designated biogenic [2]. Biogenic amines (BA) are basic nitrogenous compounds with low molecular weight formed mainly by decarboxylation

of amino acids or by amination and transamination of aldehydes and ketones [3]. The chemical structure of BAs can either be: aliphatic (putrescine, cadaverine, spermine, spermidine); aromatic (tyramine, phenylethylamine); heterocyclic (histamine, tryptamine) [3].

BAs are present in many different foods and beverages, although their concentrations vary widely between and even within food types. In addition, they may not be equally distributed within foods [3]. BA may be of endogenous origin at low concentrations in fresh foods such as fruits and vegetables, however high concentrations have been found as the result of uncontrolled microbial enzymatic activity [5]. Amino acid decarboxylation is the most common mode of synthesis of amines in foods [1]. In this way, the synthesis and accumulation of BA in food is dependent on the specific bacterial strain(s) present, the level of decarboxylase activity, the availability of the amino acid substrate and environmental conditions that allow both for the necessary enzyme action and bacterial growth [4]. Combinations of these factors influence the varieties and quantities of BAs present in foodstuffs. Although BAs are usually formed during a decomposition or spoilage process involving formation of free amino acids through proteolysis together with bacterial production and action of amino acid decarboxylases [1]. Foods likely to contain high levels of BA included fish and fish products, dairy products, meat and meat products, fermented vegetables, soy products and alcoholic beverages such as wine and beer [2]. The biogenic amines are also frequently found in high concentration in food and its byproducts subjected to deliberate or accidental bacterial contamination, like fermented and spoiled foods respectively [1]. For this reason, the content of BAs in foods is considered as an indication of its quality, being tracked in plenty of foods processing to monitor the level of microbiological contamination.

Biogenic amines are needed for many physiological function in human and animals, however toxicological effects such as headache, rash, diarrhea, respiratory distress, heart palpitation, hypertension or hypotension may occur if BAs are ingested in excessive amounts with consumption of food heavily contaminated or when the natural mechanism for their catabolism are inhibited or genetically deficient [1]. Based on the mode of action, biogenic amines can be differentiated into vasoactive and psychoactive amines [9]. Psychoactive amines influence neural transmitters in the central neural system, while vasoactive amines act either directly or indirectly on the vascular system. Histamine, putrescine and cadaverine are psychoactive amines, while tyramine, tryptamine and phenylethylamine are vasoactive amines. Histamine poisoning (scombroid poisoning) is a worldwide problem that occurs after the consumption of food containing psychoactive BAs, particularly histamine at concentrations higher than 500 ppm. Histamine poisoning manifests itself as an allergen-type reaction characterized by difficulty in breathing, itching, rash, vomiting and fever [12]. The action of vasoactive presser tyramine, tryptamine and phenylethylamine can cause hypertensive crisis in individuals. The physiological effect of tyramine include peripheral vasoconstriction, increases the cardiac output, increases respiration, elevated blood sugar, releases noradrenaline and causes migraine [5,9]. Tryptamine may lead into depression and hepatic encephalophaty [10]. Phenylthylamine can cause migraine, dizzines and increase the blood pressure [11]. Several biogenic amines are also precursor of carcinogenic compounds, such as N-nitrosamines [1,5]. For example, putrescine and cadaverine can be converted into pyrrolidine and piperidine, respectively, from which carcinogenic nitrosopyrrolidine and nitrosopiperidine are formed by heating [8].

Biogenic amine formation can be controlled through inhibiting microbial growth or inhibiting the decarboxylase activity of microbes. The prevention of biogenic amine formation in food has, therefore, been achieved using temperature control, using high-quality raw material, good manufacturing practice, the use of nonamine forming (amine-negative) or amine oxidizing starter cultures for fermentation, the use of enzymes to oxidize amines [13], the use of microbial modeling to assess favorable conditions to delay biogenic amine formation, packaging techniques and food additives [14]. Biogenic amine formation is temperature dependent [1], and it is decreased at low temperatures through inhibition of microbial growth and the reduction of enzyme activity [15]. High-temperature treatments can also be used to extend the shelf life of food. A thermal regime designed to kill the bacterial species responsible for BAs formation and can prevent the subsequent formation of BAs. However, if recontamination and temperature abuse occurs after thermal processing, histamine formation may still occur in the thermally processed product. Besides, biogenic amines are reported as heat stable compounds and cooking or prolonged exposure to heat will not eliminate the toxin so applying heat after BAs have formed in the product will not ensure its safety [16]. Despite this like most traditional processes of preservation and improvement of food involves heating or cooling, in general, the established methods for the control of biogenic amines are based on temperature variations [4].

Over years toxicological innocuity, nutritional adequacy and microbiological safety of irradiated food has been carefully evaluated by an unprecedented width of research and testing. All scientifically acceptable evidence resulted from those studies supports the safety of irradiated food for consumption [17]. Because of this the FAO/IAEA/WHO Expert Committee on Food Irradiation concluded that the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard and further introduces no special nutritional or microbiological problems [18]. The Brazilian law goes further when it states in the RDC n^{o} 21, 26 January 2001, that ionizing radiation can be used to treat food without any fixed limits to the dose applied, just that the quality of food is ensured by the use of a minimum dose enough to reach the intended purpose and a maximum dose lower than that would compromise the functional properties and sensory attributes or the food [26]. In this way, food irradiation has been used in many countries for the purposes of inhibition of sprouting, destruction of food borne insects and parasites, delay of physiological ripening, extension of shelf life or improvement of the technological properties of food [19]. Irradiation is effective in reducing microorganisms and viruses, and is known as a good method for inactivating pathogens in food materials [20]. Furthermore, besides the sanitary purpose, irradiation technology in new trials is applied to induce radiolysis and reduce the content of toxic compounds in foods like carcinogenic nitrosamine and nitrite [21] and BAs in an aqueous model system [22].

Usually during food irradiation there are compounds that break down more easily to form radiolytic products than likely trigger both oxidation and reduction reactions. For example, water's radiolytic products are notably the hydroxyl radical, a powerful oxidizing agent and aqueous electron or hydrogen atom, a reducing agent [23]. Other compounds have a similar behavior and therefore they are also suitable for the construction of model systems. Methanol has these characteristics. It is radio-sensible and produces when it breaks hydrogen atom (reducing agent) and CH_3-$O^{\cdot}$ (oxidizing agent). Therefore, this study was designed to verify the effect of different gamma irradiation doses (1, 3 and 5kGy) in methanol solutions of three different biogenic amines: tryptamine, tyramine and 2-phenylethylamine and to evaluate sensitivity of these BAs by gamma irradiation.

2. MATERIALS AND METHODS

2.1. Samples Preparation

Standard BAs, 2-phenylethylamine (PHE) (98%), tryptamine (TRP) (98%) and tyramine (TYR) (99%), were purchased from Sigma-Aldrich Co. Chemical Co. (Brazil). The BAs were dissolved in methanol HPLC grade with a 100 µg/ml concentration. Samples were kept in a dark container and refrigeration to minimize external factors of degradation.

2.2. Irradiation

The irradiation of the samples occurred at the Centro Tecnológico do Exército (CTEx), located in Guaratiba, Rio de Janeiro, RJ, Brasil with a research irradiator with Cesium-137 source. The radiator is a shielded cavity and has the useful volume of the order 100 L. The irradiator was designed and built at Brookhaven National Laboratory (USA) in 1969. The current activity of the source is approximately 45 kCi with a dose rate ranging between 1 kGy/h and 1,8 kGy/h with good uniformity. The exposure time was calculated using a program developed especially for the radiator, based on a mapping dosimetric, which takes into account the current activity of the source, desired mean dose (Gy), the diameter or height, density and geometry of the sample, the high-attenuation factor and the build-up [24]. The irradiation doses in this study were 0, 1, 3 and 5 kGy.

2.2. Biogenic Amines Analysis

For analysis of BAs phenylethylamine, tyramine and tryptamine were used gas chromatography (GC) associated with a mass detector (MS). Chromatographic conditions were: interface temperature 250 °C, mobile phase helium, manual injection, injector "split-splitless" with split rate of 1/5 and flow rate of 1.00 mL/min. The heating was linear between 40 °C to 300 °C with 10 °C/min rate, remaining at maximum temperature for two minutes. The chromatographic separation was done with Rtx-5MS column with dimensions 30m x 0.25 mm and 0.25 microns thick, the stationary phase was 5% diphenyl - 95% dimethylpolysiloxane. The identification and quantification of compounds of interest was performed with a mass selective detector in SCAN mode for m/z 15 to 300 [25].

3. RESULTS

3.2 Chromatographic profiles comparison

The figures 1, 2 and 3 show the chromatographic profiles comparison of the three studied BAs samples in these conditions of analysis.

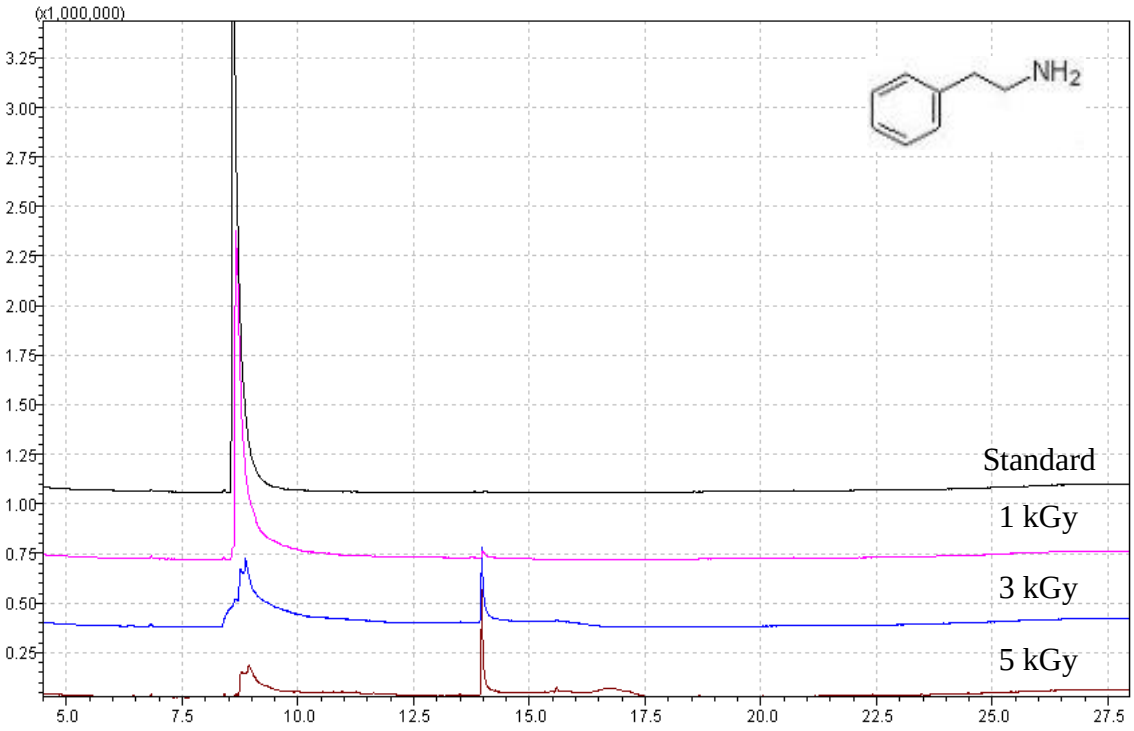

Figure 1. Comparison of Phenylethylamine solutions – Standard, 1kGy, 3kGy and 5kGy – SCAN mode, Methanol as solvent.

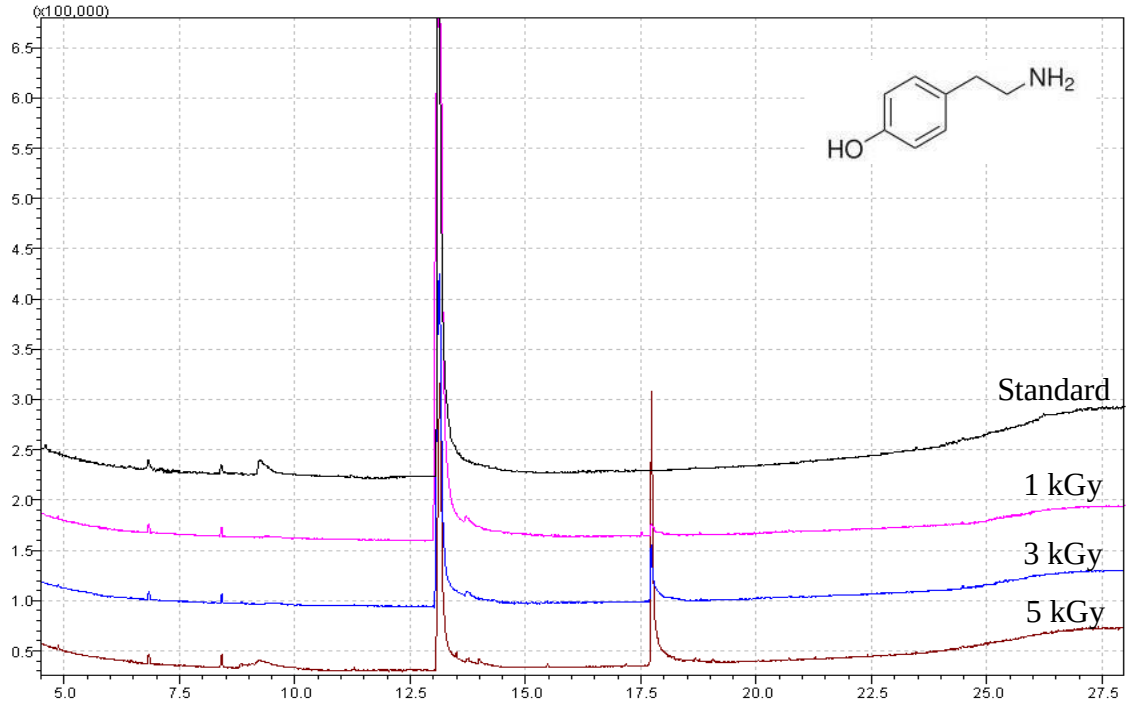

Figure 2. Comparison of Tyramine solutions – Standard, 1kGy, 3kGy and 5kGy – SCAN mode, Methanol as solvent.

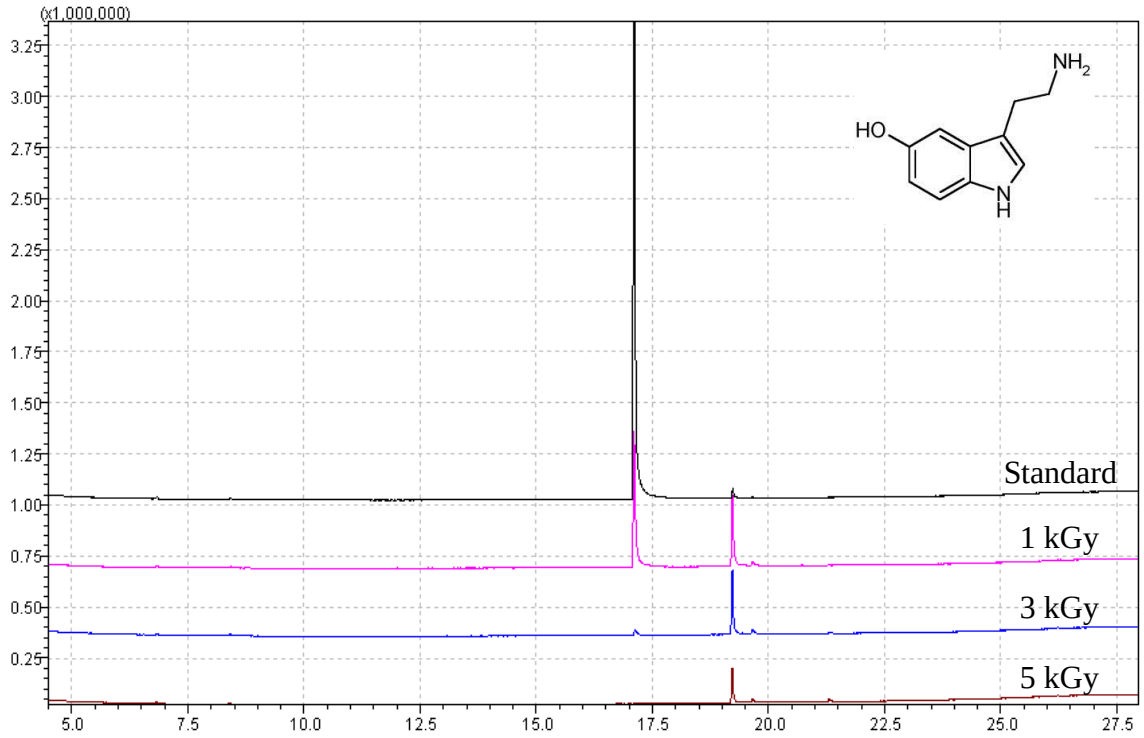

Figure 3. Comparison of Tryptamine solutions – Standard, 1kGy, 3kGy and 5kGy – SCAN mode, Methanol as solvent.

3.2 Degradation rate of biogenic amines

The contents of BAs dissolved in methanol were measured to investigate basic radiolytic characteristic of BAs after gamma irradiation (Table 1).

Table 1. Changes in BA content after gamma irradiation (unit: μg/ml)

Dose (kGy)	Time (min)	TRP[a]	PHE[b]	TYR[c]
0	0	100.00	100.00	100.00
1	34.29	15.11	79.97	80.75
3	102.85	0.88	28.90	49.89
5	171.43	0.00	15.01	39.60

a. Tryptamine; b. Phenyletilamine; c. Tyramine

The degradation of BAs by irradiation was kinetically studied according to previous studies [21, 22]. The irradiation time corresponding at each dose studied was calculated using a specific program developed for the irradiator. The correlation coefficient (R_2) was calculated from the plot of the logarithm of the concentration of BA standards versus irradiation time (Figure 4). The degradation rate constant (K, min^{-1}) was obtained from the following equation:

$$K = -\ln(CA/CA_0)/t \quad (1)$$

In this equation CA is the concentration of BA standards after irradiation and CA_0, the initial concentration of BA standards. The ratio CA/CA_0 was calculated by the ratio between the peak areas corresponding to products of interest in the chromatograms. Table 2 shows the calculated constants.

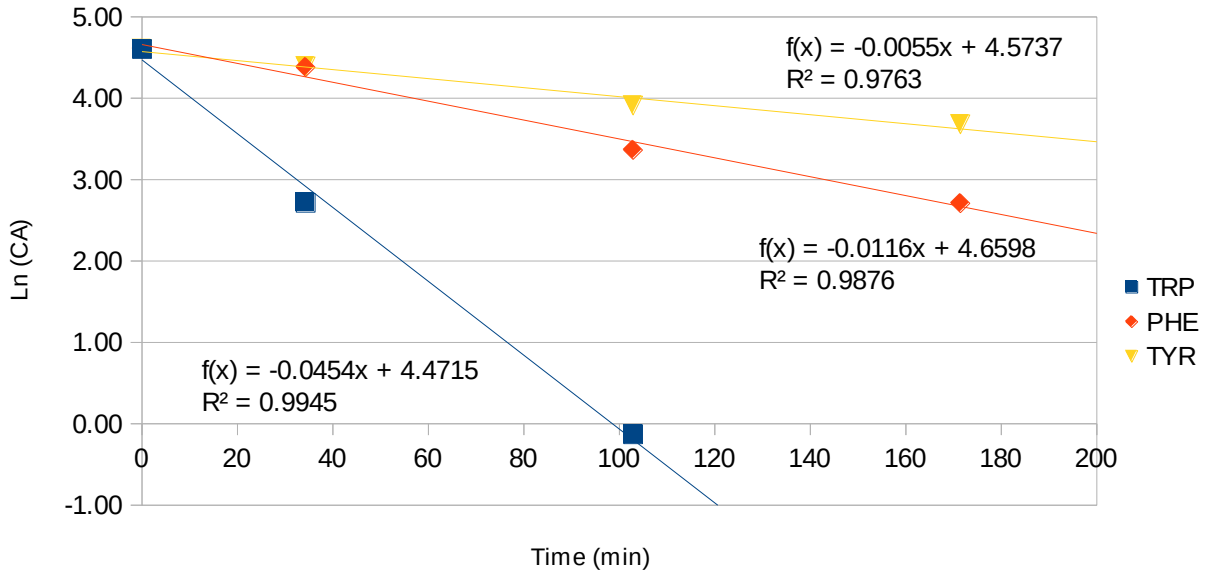

Figure 4. First-order plot for degradation of Phenylethylamine (PHE), tyramine (TYR) and tryptamine (TRP) by gamma irradiation (R_2: correlation coefficient, CA: the concentration of BA after irradiation).

Table 2. Degradation rate constants

	TYR[a]	PHE[b]	TRP[c]
K	0.0055	0.0116	0.0454

a. Tryptamine; b. Phenyletilamine; c. Tyramine

4. CONCLUSIONS

The BAs in this study were significantly reduced in a dose-dependent manner. Tryptamine was more radiosensitive being reduced by about 85% at 1kGy, 99% at 3kGy and at 5kGy TRP were completely broken down by irradiation. The phenylethylamine was reduced from its initial amount in about 20% at 1kGy, 70% at 3kGy and 85% at 5kGy. Tyramine showed

less sensitivity to gamma radiation with reduction only 20% at 1kGy, 50% at 3kGy and 60% at 5kGy. Therefore, radiolysis of BAs in a model methanol system was observed by gamma irradiation. The behavior of the studied BAs was similar to the aqueous models for TYR and PHE. TRP was an exception because it was more sensitive in this model. Thus we can conclude that more studies are needed, mainly to verify the influence of irradiation treatment in high levels BAs contaminated foods, where the influence of a complex matrix, with its various components should be considered. Furthermore, analysis of the radiolytic products from irradiated BAs and their biological effect should be determined for application on foods. However, gamma irradiation could be expected to reduce or eliminate food-poisoning BAs in food systems in future.

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