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Article

Degradation of Phenylethylamine and Tyramine by Gamma Radiation Process and Docking Studies of its Radiolytes

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Aminas biogênicas (BA) são bases orgânicas tóxicas de baixo peso molecular, com estruturas alifáticas ou heterocíclicas, que podem ser encontradas em vários alimentos. O consumo de alimentos contendo grandes quantidades de BA pode resultar em reações alérgicas, erupções cutâneas, vômito e hipertensão. Além disso, as BA são também possíveis precursores de carcinógenos. No presente estudo nós avaliamos o efeito de diferentes doses de irradiação gama em soluções metanólicas e aquosas das BA feniletilamina e tiramina. Nossos melhores resultados mostraram que, na dose de 5 kGy (unidade SI utilizada para a medição de dose absorvida de radiação ionizante), foi possível reduzir o conteúdo dessas duas BA em até 85 e 60% respectivamente, sugerindo que a irradiação pode ser uma eficiente ferramenta para a sua degradação. Estudos adicionais por ancoramento molecular sugerem que os radiólitos dessas BA, produzidos durante o processo de irradiação, teriam mais afinidade pelas enzimas humanas detoxificadoras monaminoxidases tipo A e B (MAO-A e MAO-B) sendo, portanto, menos tóxicos que seus precursores.

Biogenic amines (BA) are toxic low molecular weight organic bases with aliphatic or heterocyclic structures that can be found in several foods. The consumption of food containing large amounts of BA can result in allergic reactions, rash, vomiting, and hypertension. Besides, BA are also known as possible precursors of carcinogens. In the present study we evaluated the effect of different gamma irradiation doses on methanol and water solutions of the BA phenylethylamine and tyramine. Our best results showed that, at a dose of 5 kGy (SI unit used for measurement of absorbed dose of ionizing radiation), it was possible to reduce the content of these two BA up to 85 and 60%, respectively, suggesting that the use of the irradiation process can be an efficient tool for its degradation. Further docking studies also suggested that the radiolytes produced in the irradiation process have more affinity for the human detoxifying enzymes monoaminoxidases type A and B (MAO-A and MAO-B) being, therefore, less toxic than its precursors.

Keywords: biogenic amines, gamma irradiation, food decontamination

Introduction

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.[1] They can be aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine)[1] and are present in many different food types and beverages at different concentrations. When at low concentrations, BA may be of endogenous origin in fresh foods such as fruits and vegetables. However, at high concentrations, they are usually the result of uncontrolled microbial enzymatic activity.[2] So, the synthesis and

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accumulation of BA in food is dependent on the presence of specific bacterial strain(s), the level of decarboxylase activity, the availability of the amino acid substrate and environmental conditions that allow for both the necessary enzyme action and bacterial growth.[3] Foods likely to contain high levels of BA include fish and fish products, dairy products, meat and meat products, fermented vegetables, soy products and alcoholic beverages, such as wine and beer.[4] BA are also frequently found in high concentrations in food and its byproducts when subjected to deliberate or accidental bacterial contamination, like fermented and rotten foods.[5] For this reason, the content of BA in foods is considered an indicator of its quality, being tracked in most of the food processes to monitor the level of microbiological contamination.

BA are needed for many physiological functions in human and animals, however, toxicological effects such as headache, rash, diarrhea, respiratory distress, heart palpitation, hypertension or hypotension may occur if they are ingested in excessive amounts.[5] Based on the mode of action, BA can be differentiated into vasoactive and psychoactive amines.[6] Psychoactive amines influence neural transmitters in the central nervous 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 BA, particularly histamine, at concentrations higher than 500 ppm. Several BA are also precursors of carcinogenic compounds, such as *N*-nitrosamines.[2,5] For example, putrescine and cadaverine can be converted into pyrrolidine and piperidine, respectively, from which the carcinogenic compounds nitrosopyrrolidine and nitrosopiperidine are formed by heating.[7] BA formation can be controlled through inhibiting microbial growth or inhibiting decarboxylase activity.

The prevention of BA formation in food has been achieved using temperature control, high-quality raw material, good manufacturing practices, the formation of non-amine (amine-negative) or amine oxidizing starter cultures for fermentation, the use of enzymes to oxidize amines,[8] the use of microbial modeling to assess favorable conditions to delay BA formation, packaging techniques and food additives.[9] However, if recontamination and temperature abuse occurs after thermal processing, histamine formation may still occur in the thermally processed products. Besides, BA are reported as heat stable compounds and cooking or prolonged exposure to heat will not eliminate these toxins. Therefore, just applying heat after BA formation in the product will not ensure its safety.[10]

Irradiation is effective in reducing microorganisms and is known as a good method for inactivating pathogens in food materials.[11] Furthermore, besides sanitary purposes, irradiation technology in new trials is applied to induce radiolysis and reduce the content of toxic compounds like carcinogenic nitrosamine.[12] Usually during food irradiation some compounds break down more easily to form radiolytic products likely to trigger both oxidation and reduction reactions. The hydroxyl radical from water's radiolytic products, for example, is a powerful oxidizing agent while the hydrogen atom is a reducing agent.[13] Other compounds have a similar behavior and, therefore, they are also suitable for the construction of model systems. Methanol has these characteristics. Its radiolytic products are $CH_3–O^{\bullet}$ (oxidizing agent) and the hydrogen atom (reducing agent). Therefore, this study was designed to verify the effect of different gamma irradiation doses (1, 3 and 5 kGy) in aqueous and methanol solutions of phenylethylamine and tyramine.

Experimental

Sample Preparation

Standard phenylethylamine (98%), and tyramine (99%), were purchased from Sigma-Aldrich Chemical Co. (Brazil). These compounds were dissolved in solutions of HPLC grade methanol and ultra-pure water at 100 μg mL^{-1} and the samples were kept in a dark container under refrigeration to minimize external factors of degradation.

Irradiation

Samples were irradiated at the Brazilian Army Technological Center, located in Guaratiba, Rio de Janeiro-RJ, Brazil, in a research irradiator with ^{137}Cs source. The radiator is a shielded cavity and has a useful volume of 100 L and was built at the 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^{-1} and 1.8 kGy h^{-1} with good uniformity. The exposure time was calculated using a program developed especially for the radiator, based on a mapping dosimetry, which takes into account the current activity of the source, desired mean dose (in Gy), the diameter or height, density and geometry of the sample, the high-attenuation factor and the build-up.[14] In average the exposure time of the samples to irradiation was 34 min *per* kGy. Thus the samples irradiated at 3 and 5 kGy had exposure times of 102 and 170 min respectively.

Analysis of the BA

Gas chromatography-mass spectrometry (GC-MS)

Phenylethylamine and tyramine were analyzed in a GC-MS instrument (Shimadzu QP2010, Tokyo, Japan). Chromatographic conditions were: interface temperature of 250 °C, mobile phase He, manual injection, injector "split-splitless" with split rate of 1/5 and flow rate of 1.00 mL min^{-1}. The heating was linear between 40 to 300 °C with 10 °C min^{-1} rate, remaining at maximum temperature for two minutes. The chromatographic separation was done with Rtx-5MS column with dimensions 30 m × 0.25 mm × 0.25 mm and the stationary phase was 5% diphenyl/95% dimethylpolysiloxane. The identification and quantification of compounds of interest were performed with a mass selective detector in scan mode for *m/z* 15 to 300.[15]

The methanolic solutions were analyzed by direct injection without any previous treatment. Aliquots of 300 µL of each aqueous solution were separated and kept under water bath at 60 °C and submitted to a continuous flow of N_2 for about 15 min until evaporation of the water.[16]

Analysis of the BA solutions by electrospray ionization mass spectrometry (ESI-MS)

ESI-MS analyses were performed by direct injection using syringe pump at a flow of 5 µL min^{-1} under the same conditions for each sample group. The nebulizing gas flow was 500 L h^{-1} at 140 °C and the cone gas was adjusted to 50 L h^{-1} at 100 °C. The capillary and cone voltages were 4000 V and between 15 and 30 V, respectively. The acquisition rate of the mass analyzer (a QTOF mass spectrometer from Waters with a lockmass system) was fixed at 1.0 s with 0.4 s of delay between scans. The analytes were acquired using fixed spray to ensure mass precision. The calibration of the equipment was performed with a 0.1% m/v phosphoric acid solution.

In our experiments the collision gas used was Ar and the collision energies ranged between 15 and 25 eV. Ionization in the positive mode was used for the analysis of the aqueous and methanolic solutions (100 µg mL^{-1}) of phenylethylamine and ionization in the negative mode was used for the analysis of the aqueous and methanolic solutions (100 µg mL^{-1}) of tyramine. The experiments were performed only with the control and the samples irradiated at 5 kGy for verification of the exact masses of the radiolytic products formed.

The MS experiments were performed by monitoring the protonated molecule $[M + H]^+$ for the positive mode and the deprotonated molecule $[M - H]^-$ for the negative mode of the analyzed compounds.

Docking studies

The structures of the BA and its radiolitic products were built and optimized in the PC Spartan PRO® software[17] and its partial charges were calculated through single point energy calculations using the semi-empiric method RM1.[18]

The crystallographic structures of human enzymes MAO-A, complexed with harmin,[19] and MAO-B, complexed with phenylcyclopropilamine and the cofactor flavin adenine dinucleotide (FAD),[20] respectively, were downloaded from the Protein Data Bank (PDB)[21] under the codes: 2Z5X (Resolution = 2.2 Å and R factor = 0.204) and 2XFU (Resolution = 1.7 Å and R factor = 0.189). The crystals were imported exactly as they are in PDB,[21] including its ligands and crystallographic water molecules. Considering that all these enzymes are active as monomers, just the monomeric units were used for the docking studies.

The software Molegro Virtual Docker® (MVD)[22] was used to perform the docking studies following a protocol used in former studies.[23,24] For this task each one of the studied enzymes was first prepared through the detection of cavities, selection of the water molecules relevant for the study, flexibilization of the active site residues and definition of the restriction areas for the dockings. Table 1 summarizes the docking parameters used.

The docking protocol used was validated by re-docking of the ligands present in each enzyme. The best conformations of each studied compound were chosen according to their docking score and superposition to the ligands present inside each enzyme.

Statistical analysis

The chromatographic data obtained for each dose were submitted to the analysis of variance and averages

Table 1. Parameters for the docking studies

Enzyme	Flexible residues	Volume of the cavity / Å^3	Coordinates of the restriction area / x, y, z	Number of water molecules considered
MAO-A	All residues at 8 Å from harmine (64)	207.36 involving harmine and FAD	Sphere with 8 Å of radius and centered at x = 40.48; y = 26.72; z = −13.52	8
MAO-B	All residues at 12 Å from phenylcyclopropilamine (232)	201.73 involving phenylcyclopropilamine and FAD	Sphere with 5 Å of radius and centered at x = 54.86; y = 150.79; z = 25.04	52

(ANOVA/MANOVA) at a level of confidence of 95%. Once calculated, the values of averages and standard deviations related to each dose were compared to verify if alterations related to the doses applied were really meaningful. Besides, for the calculation of the calibration curves, we used an internal prediction of the values of 95%. The values and graphs were calculated using the STATISTICA® and Microsoft Excel® softwares.

Results and Discussion

Irradiation studies

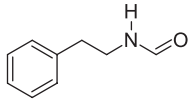
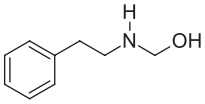
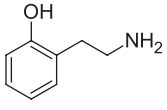
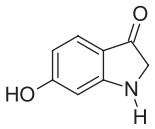
Methanolic solutions of irradiated phenylethylamine

In order to quantify the phenylethylamine samples by GC-MS, we first built a calibration curve through the injection of 3 different dilutions (25, 50 and 200 mg mL^{-1}) of the methanolic standard solution in duplicate and the dilution at 100 mg mL^{-1} in triplicate. The calibration curve obtained presented an R^2 factor of 0.9888 and was, therefore, considered suitable.

The GC-MS analyses had the goals of verifying the reduction of the contaminant content and identify the appearance of peaks indicative of new compounds formed by irradiation. The chromatograms obtained for the methanolic solutions submitted to different doses are presented as Supplementary Information Figure S1A, where it is possible to notice a reduction of the phenylethyamine peak with the increasing of the irradiation dose. The relationship between the found concentrations and the applied doses can be seen in the plot of Figure S2A, where the red region includes a range of concentration that can be expected, with 95% of confidence, for both the control samples and the dose of 1 kGy. This means that the reduction obtained at 1 kGy in the content of phenylethylamine is not significant enough to be different from the control sample. However, at 3 kGy, the reduction is noticeable and at 5 kGy the inactivation is even more effective.[25]

Analysis of the mass spectra for each one of the chromatographic peaks found suggest that the radiolytic products can present structures with carbonyl groups bonded to the N atom of the aliphatic chain. The chromatographic peaks chosen for comparison in the mass spectra, with the respective retention times, are described in Table 2. The similarities observed regarding the mass spectra from NIST library were above 90 for all compounds.

Table 2. Main peaks in the chromatograms of the methanolic and aqueous solutions of phenylethylamine irradiated at different doses. It was not possible to identify peak 1

Analytes	t_R / min	Average area of the peak at each dose			
		Standard	1 kGy	3 kGy	5 kGy
		Methanolic solutions			
Phenylethylamine	6.09	14189881	12064530	3763125	422820
Peak 1 (Unidentified)	6.42	373926	8319417	13108564	11971882
Peak 2 	11.49	–	91695	769412	4979638
Peak 3 	13.08	–	–	116970	1079850
		Aqueous solutions			
Phenylethylamine	6.14	5488291	1761162	425543	368839
Peak 1 	10.76	–	54822	69598	291373
Peak 2 	12.63	–	–	94584	238516

The electrospray ionization high-resolution mass spectrometry (ESI-HRMS) analysis was performed after injection of the standard methanolic solution (100 μg mL^{-1}) of phenylethylamine. The ionization mode used was the positive one and the bonding formation between the H and N atoms of the BA was expected due to the free electrons of the N atom. Prior to ESI(+)-MS or ESI(−)-MS analysis, 1 μL of an aqueous solution of 0.1% ammonium hydroxide (negative mode) or formic acid (positive mode) (v/v) was added to 1 mL of each sample. MS analysis was performed in order to check the preferential profile of fragmentation with an initial selection of the ion *m/z* 122 equivalent to $[PHE + H]^+$. The results were used as a reference for the interpretation of the ESI-HRMS spectra of the irradiated methanolic solutions.

The exact masses of the radiolytic products were obtained by ESI-HRMS, so it was possible to achieve precision until the third decimal. Besides, the ionization used (ESI) is mild enough so that most of the ions in the mass spectra are related to the different components of the tested samples (protonated or deprotonated molecules). The ESI-HRMS spectra obtained for the solution irradiated at 5 kGy for 170 min is shown in Figure 1. The ion circled with a solid line refers to phenylethylamine $[PHE + H]^+$ while the ones circled with dashed lines refer to the radiolytes (suggested by GC-MS) and the ones circled with dashed and dotted lines refer to new peaks, indicative of thermally unstable compounds not observed before. The dashed square shows the two most intense ions, considered here as the main radiolytic products. The proposed structures of these compounds, together with their possible mechanistic schemes, are shown in Figure 2a. These schemes, based on the literature,[26] considered that the molecules in the excited states generated by the ionizing radiation tend to form mainly free radicals that then act on the other molecules to form different radiolytic products.

Aqueous solutions of irradiated phenylethylamine

After drying and re-suspension in methanol, the aqueous solutions of phenyltehylamine, submitted to different irradiation doses, were analyzed by GC-MS. The chromatograms obtained are shown as Figure S1B. In comparison with the methanolic solutions (Figure S1A) we can see that the peaks present a smaller intensity. This

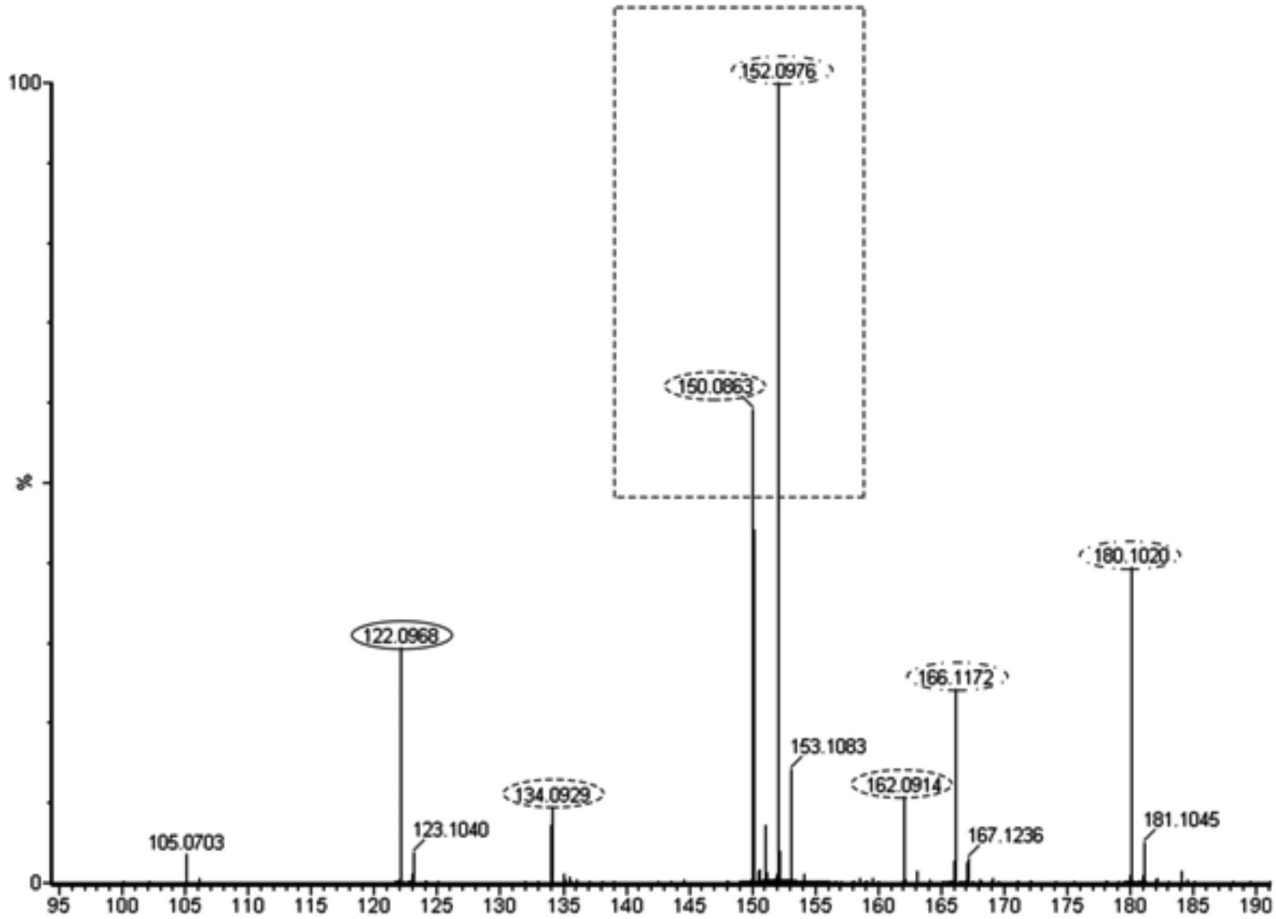


Figure 1. ESI-HRMS spectra for the methanolic solution of phenylethylamine irradiated at 5kGy for 170 min.

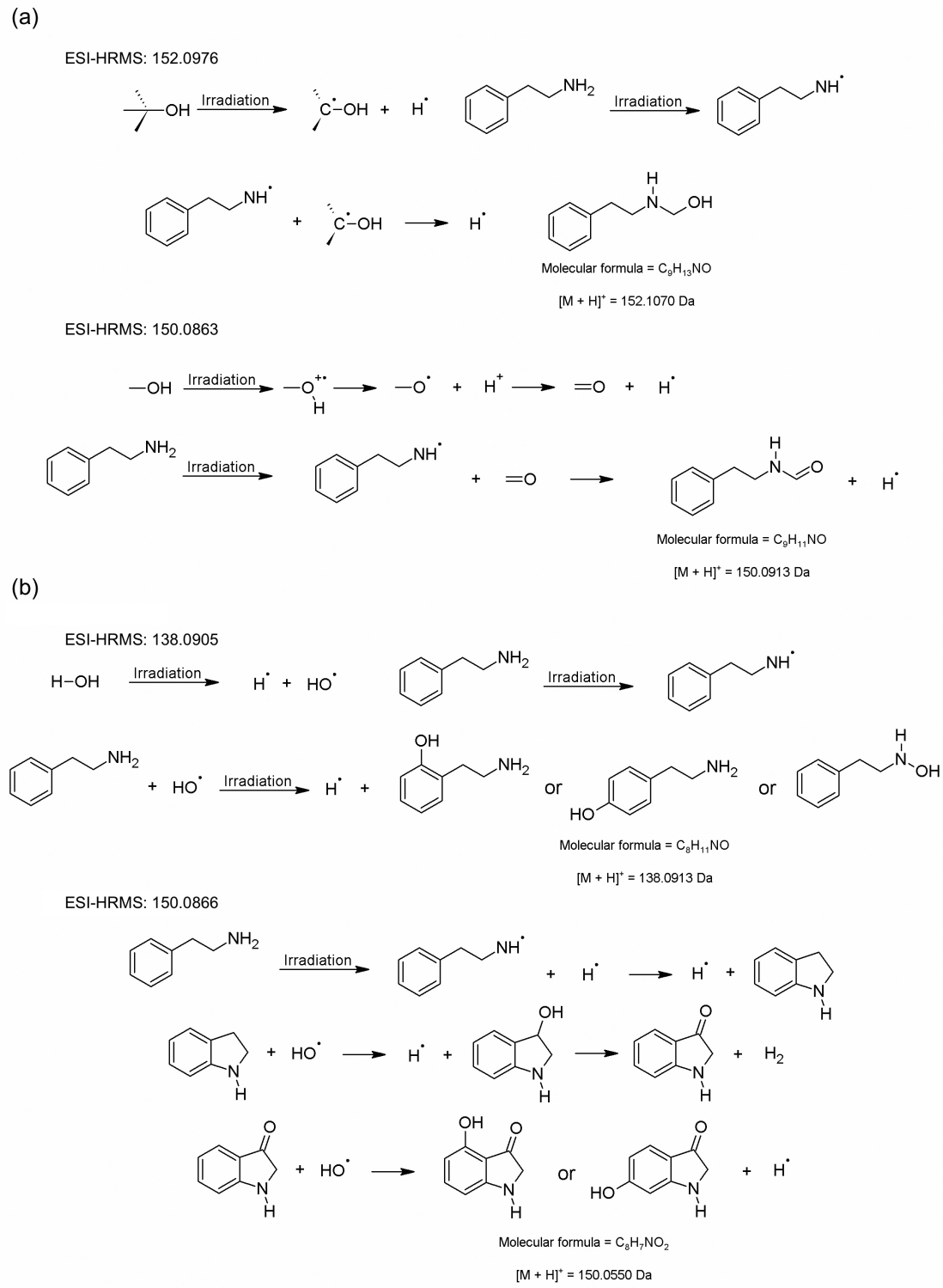


Figure 2. Proposed structures of the main radiolytic products of the methanolic (a) and aqueous (b) solutions of phenylethylamine and possible mechanistic schemes for their formation.

probably happens due to the losses in the processes of drying and re-suspension. The calibration curve showed that these losses were up to 40%.

In order to quantify the inactivation we plotted the calculated concentrations for the phenylethylamine peaks in the chromatograms *vs.* dose (Figure S2B). This graph showed that the dose of 1 kGy was enough to promote an inactivation similar to the doses of 3 and 5 kGy.

In the qualitative analysis, similarly to the methanolic solutions, the mass spectra of the distinguishing peaks

(Table 2) were taken for comparison with the library (NIST05s.LIB), indicating that probably there are cyclic structures and/or holding hydroxyl groups.

The ESI-HRMS spectra obtained for the solution irradiated at 5 kGy for 170 min is shown in Figure 3. The ion circled with a solid line refers to phenylethylamine [PHE + H] while the ones circled with dashed lines refer to the radiolytes suggested by the GC-MS. The intensities of the signals suggest that small amounts of radiolytic products were formed. This is in accordance with the GC-MS results. The dashed square shows the two most intense ions, considered here as the main radiolytic products. The proposed structures of these compounds, together with their possible mechanistic schemes, are shown in Figure 2b.

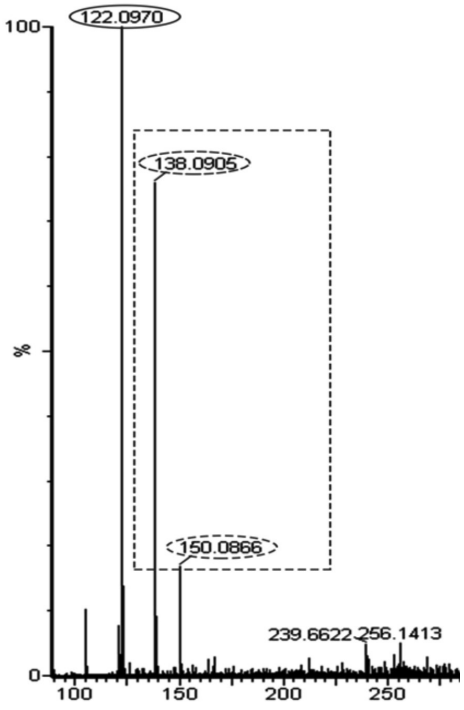


Figure 3. ESI-HRMS spectra for the aqueous solution of phenylethylamine irradiated at 5kGy for 170 min.

Methanolic solutions of irradiated tyramine

In order to quantify the tyramine samples by GC-MS, we first built a calibration curve with 12 points (3 for 100 mg mL^{-1}, 2 for the other concentrations and 1 at zero). The calibration curve obtained (data not shown) presented an R^2 factor of 0.9846.

The reduction of the tyramine content and the appearance of new peaks were monitored by GC-MS. The chromatograms obtained for the methanolic solutions submitted to different irradiation doses are shown as Figure S3A. The quantitative analysis showed that the reduction of the tyramine concentration is basically the same at the doses of 3 and 5 kGy (Figure S4A).

The qualitative analysis of the mass spectra of the main peaks of interest in the irradiated samples showed carbonylated structures bonded to the N atom of the aliphatic chain. Table S1 summarizes the data of these peaks.

The ESI-HRMS analysis for tyramine was performed with the addition of base in negative mode. In this mode lesser ions are generated because of the required departure of H from the molecules. For tyramine it is possible to use this technique because this amino acid possesses a hydroxyl group bonded to an aromatic ring. So the departure of H and the consequent generated negative charge can be stabilized by resonance. The ESI-HRMS analysis of the tyramine standard (100 μg mL^{-1} in methanol) was performed with MS analysis for preferential profile of fragmentation and monitoring of the ion *m/z* 136 $[TYR - H]^-$.

The ESI-HRMS spectra obtained in the negative mode for the solution irradiated at 5 kGy for 170 min is shown in Figure 4. The ion circled with a solid line refers to tyramine $[TYR - H]^-$ while the one circled with dashed lines refers to the radiolyte suggested by the GC-MS. This ion was related to the peak at *m/z* 164 and its possible structure and mechanism of fragmentation are shown in Figure 5a.

Aqueous solutions of irradiated tyramine

The chromatograms for the irradiated aqueous samples of tyramine are shown as Figure S3B. The reduction in the control peak compared to the methanolic solutions was of 22%, indicating a small loss in the processes of drying and re-suspension in methanol. The reduction of the peaks related to tyramine, with the increasing in the dose, can also be observed. However, there was no detection of the radiolytic products by GC-MS. The reduction in the tyramine concentration implicates in a lower disponibility of tyramine in the irradiated solutions, explainable only by chemical transformations. This suggests that the products formed are not detectable by this technique and probably are thermally unstable. Table 2 shows the reduction in the average areas of the peaks related to tyramine and the variation of the tyramine concentration with the dose is shown as Figure S4B. It can be seen that the dose of 1 kGy is not sufficient for a significant reduction of tyramine in aqueous solutions. The irradiation at 3 and 5 kGy, on the other hand, was shown to be effective in the reduction of tyramine.

The ESI-HRMS spectra obtained in the negative mode for the solution irradiated at 5 kGy for 170 min is shown in Figure 6. The region where the tyramine peak was expected is shown circled with a solid line. This suggests a great inactivation of the sample. The ions formed suggest the

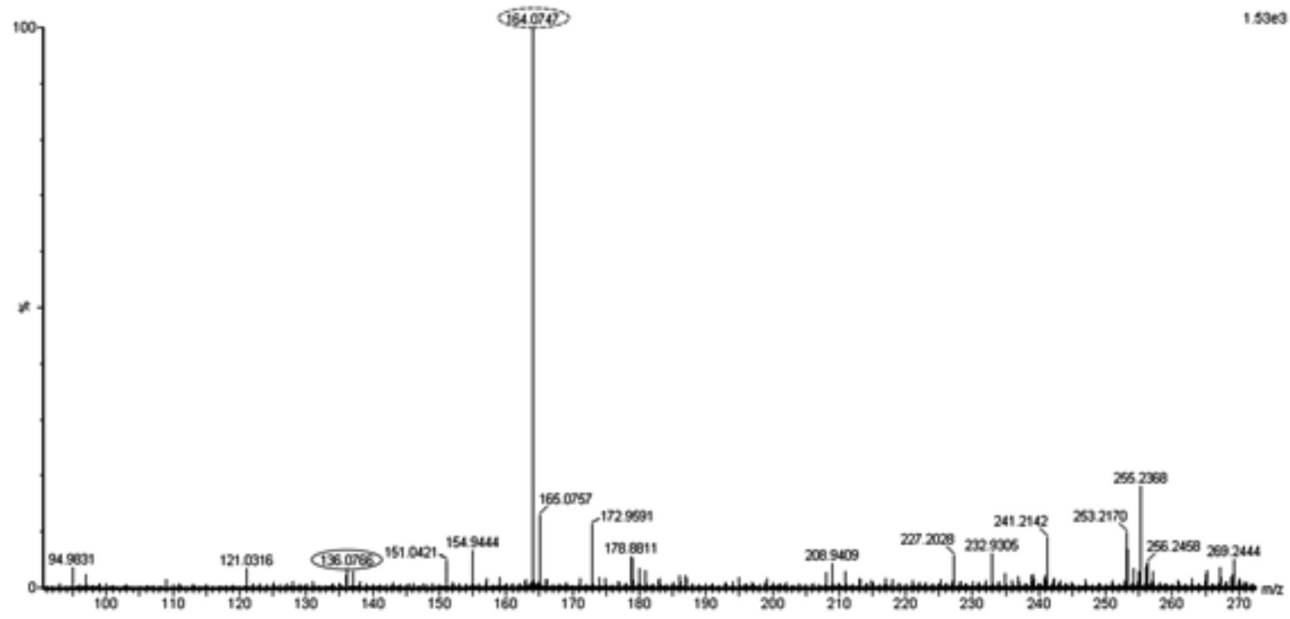


Figure 4. ESI-HRMS spectra for the methanolic solution of tyramine irradiated at 5 kGy for 170 min.

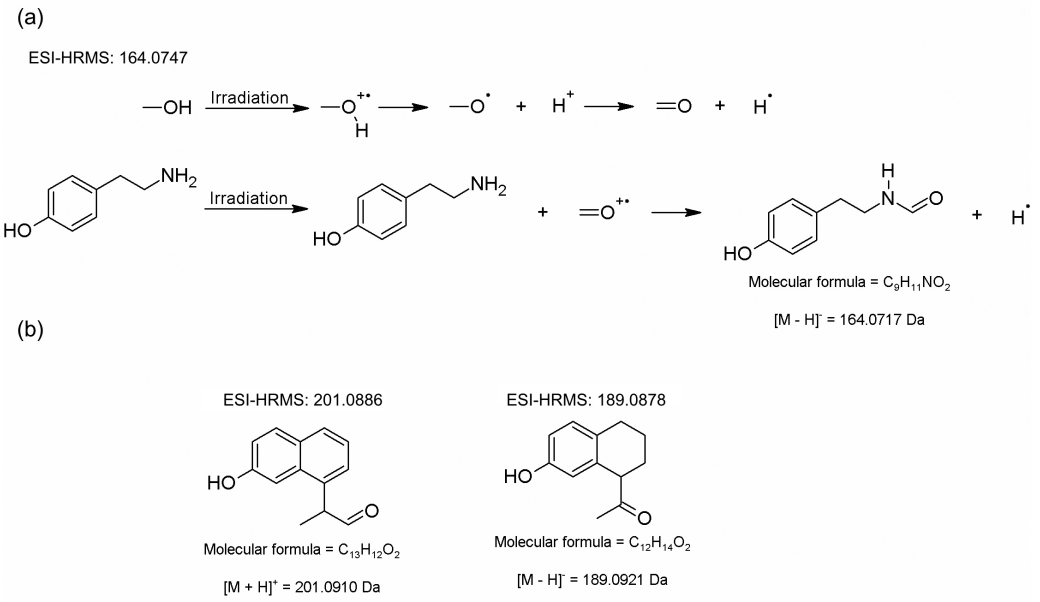


Figure 5. (a) Proposed structures of the main radiolytic products of the methanolic solution of tyramine and possible mechanistic schemes for their formation and (b) proposed structures of the radiolytes of tyramine irradiated in aqueous solution according to the exact mass encountered.

occurrence of complex reactions involving break of the carbonic chain and the leaving of the N atom. Figure 5b presents structural proposals for the ions circled with dotted and dashed lines and the molecular formulae related to the exact masses encountered.

Docking studies

The goal of the docking studies was to analyze the possible interactions of the radiolytic products inside the potential human body targets of the BA studied, namely the enzymes MAO-A and MAO-B. These interactions were evaluated according to the values of the docking energies and H-bonds inside the active sites of these enzymes.

The docking protocol used was validated by re-docking of the ligands found inside the crystallographic structures of MAO-A and MAO-B. These ligands were harmine for MAO-A and the cofactor FAD for MAO-B and the random mean square deviation (RMSD) values found were 0.452 and 0.840 Å, respectively. Keeping in mind that an RMSD value under 2.000 Å is considered acceptable,[27,28] these results validate the docking protocol used.

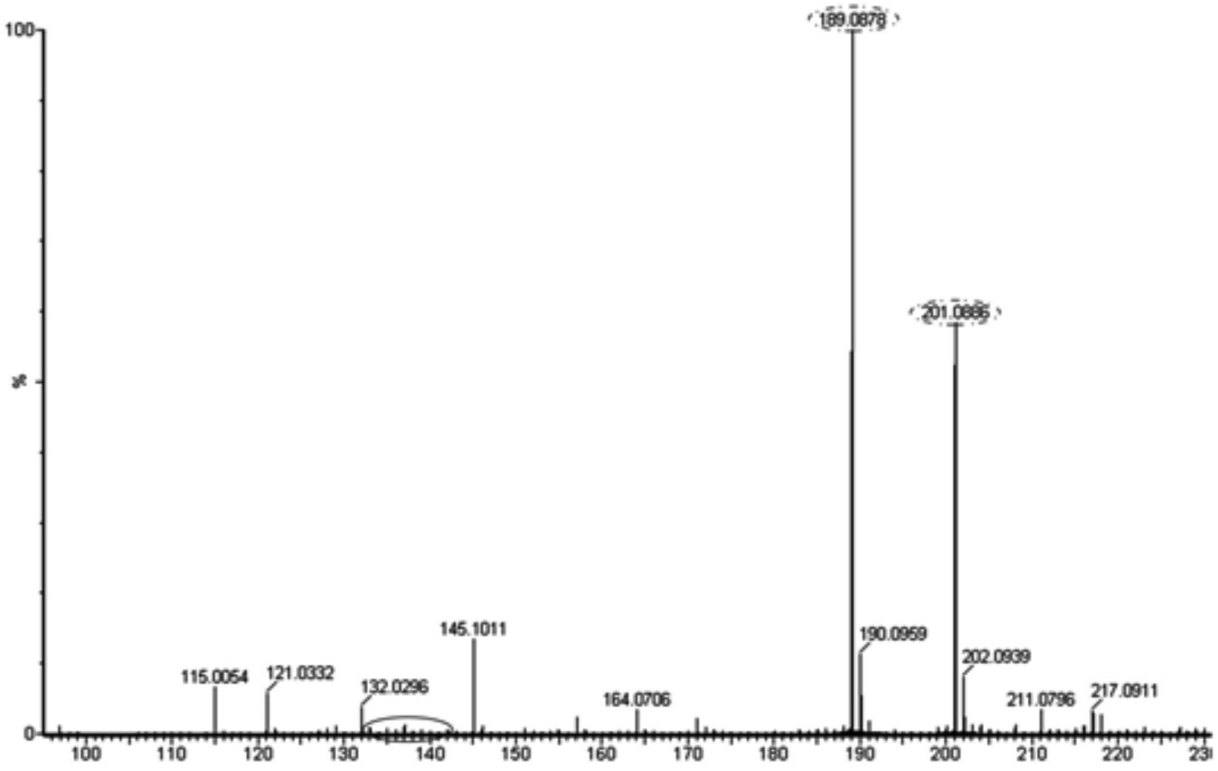


Figure 6. ESI-HRMS spectra for the aqueous solution of tyramine irradiated at 5 kGy for 170 min.

Interactions of phenylethylamine and its radiolytic products with MAO-B

According to the literature, MAO-B is the main enzyme responsible for the detoxification of phenylethylamine in the human body.[29] Therefore, docking studies were performed in order to evaluate the affinities of this BA and its radiolytic products for this enzyme. The results obtained (summarized in Table 3) show that all 4 radiolytes present energy values lower than phenylethylamine inside MAO-B. This suggests that the radiolytic products have more affinity for the enzyme than phenylethylamine and could be detoxified faster, remaining less time in the organism and inducing less toxic effects. Thus, the ionizing irradiation, besides reducing the content of phenylethylamine, has also the potential of transforming it in less toxic products.

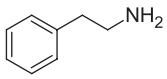
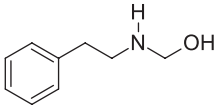
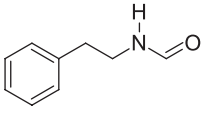
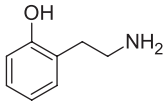
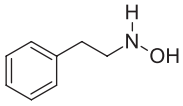
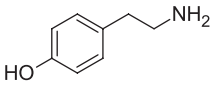
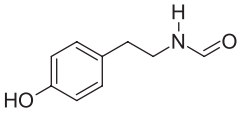
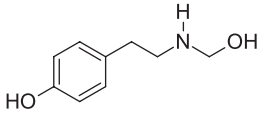
Interactions of tyramine and its radiolytes with MAO-A

Considering that MAO-A is the principal enzyme acting in the detoxification of tyramine in the human body[4] we also performed the docking studies of tyramine and its radiolytes inside this enzyme. The results obtained are summarized in Table 3 and show that, in the same way as for phenylethylamine, the radiolytic products also showed better docking values than tyramine for MAO-A. These results also suggest that the radiolytic products of tyramine have more affinities for MAO-A and, therefore, would be detoxified faster.

Conclusions

In this work we evaluated the behavior of phenylethylamine and tyrosamine when submitted to different doses of gamma irradiation in aqueous and methanolic solutions. Results showed that, in general, even at the lower dose of 1 kGy, reduction of the amines occurred despite not in significant amounts. The dose of 3 kGy promoted an effective deactivation for most of the systems studied and the dose of 5 kGy was able to promote the complete neutralization in some systems. However, in most cases there were no significant differences between the doses of 3 and 5 kGy. We also observed a major inactivation in methanolic systems than in aqueous systems. The characterization of the possible radiolityc products pointed to 3 main chemical modifications imposed by the irradiation in the systems studied: oxidation, mainly with the formation of carbonyl and hydroxyl groups, cyclization and breaking of bonds, preferentially in the ramifications. These chemical modifications are similar to the ones occurring in the detoxification of these toxins inside the human body, suggesting that the radiolytic products are less toxic than their precursors. The docking studies corroborated these findings. In both cases the radiolytes presented better docking values inside the enzymes responsible for the detoxification of these BA inside the body. This suggests

Table 3. Docking results for phenylethylamine, tyramine and the radiolytes inside MAO-B and MAO-A

Ligand	MolDockScore / (kcal mol^{-1})	Interaction / (kcal mol^{-1})	H-Bond / (kcal mol^{-1})	Interacting residues
Docking results of phenylethylamine and its radiolytes inside MAO-B				
 Phenylethylamine	–61.339	–63.172	–5.000	Tyr188, Gly434
 Radiolyte 1	–69.875	–68.376	–3.965	Tyr60, Met436, 1 H_2O
 Radiolyte 2	–78.239	–73.925	–3.415	Tyr60, Met436, 2 H_2O
 Radiolyte 3	–66.860	–67.144	–5.000	Tyr60, Tyr435, 1 H_2O
 Radiolyte 4	–69.059	–63.416	–2.500	Tyr60, 2 H_2O
Docking results of tyramine and its radiolytes inside MAO-A				
 Tyramine	–70.123	–60.593	–2.187	Phe208, 3 H_2O
 Radiolyte 1	–85.175	–71.427	0.000	4 H_2O
 Radiolyte 2	–78.271	–66.771	–2.862	Phe208, Gln215, 4 H_2O

that they would be processed faster, remaining less time in the body and provoking less toxic effects.

Our results permit to conclude that the irradiation process is in fact efficient for the inactivation of toxins, leading to less toxic products. Besides, among the doses studied, 3 kGy was the lowest one to promote a satisfactory inactivation of the toxins, being, for this reason, indicated for inactivation of BA in foods.

Acknowledgments

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Supplementary Information

Supplementary Information (additional Table and Figures) is available free of charge at http://jbcs.sbq.org.br as a PDF file.

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Supplementary Information

Degradation of Phenylethylamine and Tyramine by Gamma Radiation Process and Docking Studies of its Radiolytes

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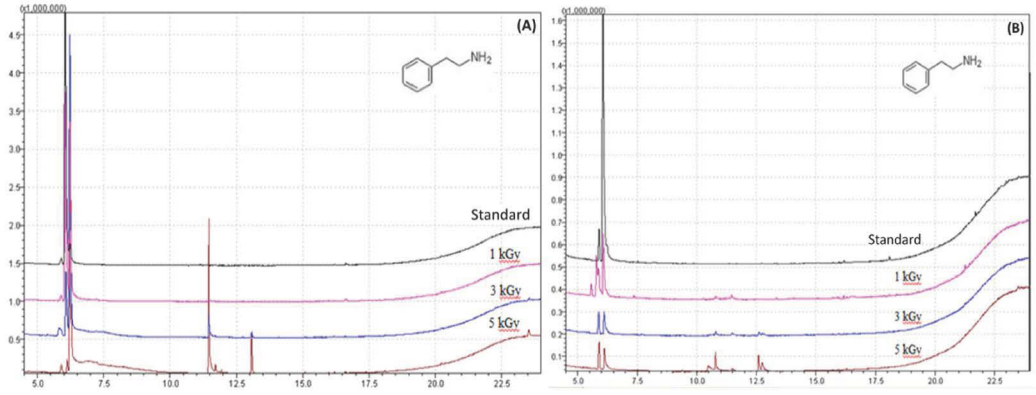


Figure S1. Chromatograms of the methanolic (A) and aqueous (B) solutions of phenylethyamine after irradiation at different doses. Mode: scan; Column: RTX-5MS (5% diphenyl/95% dimethyl polysiloxane); Ramp: 2 min, 80 °C; 10 °C min^{-1}, 80 °C to 280 °C.

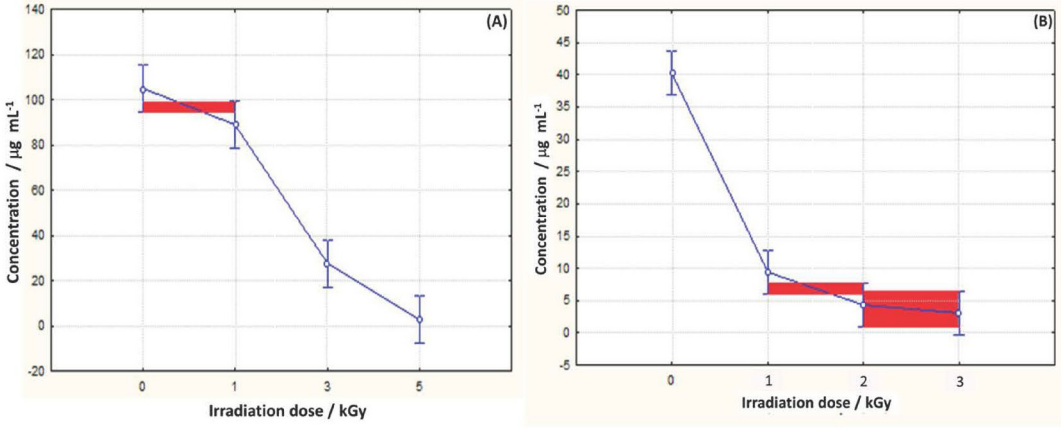


Figure S2. Plot of the phenylethylamine concentration *vs.* irradition dose for the methanolic solutions (A) and the aqueous solutions (B).

*e-mail: santoslima@ime.eb.br

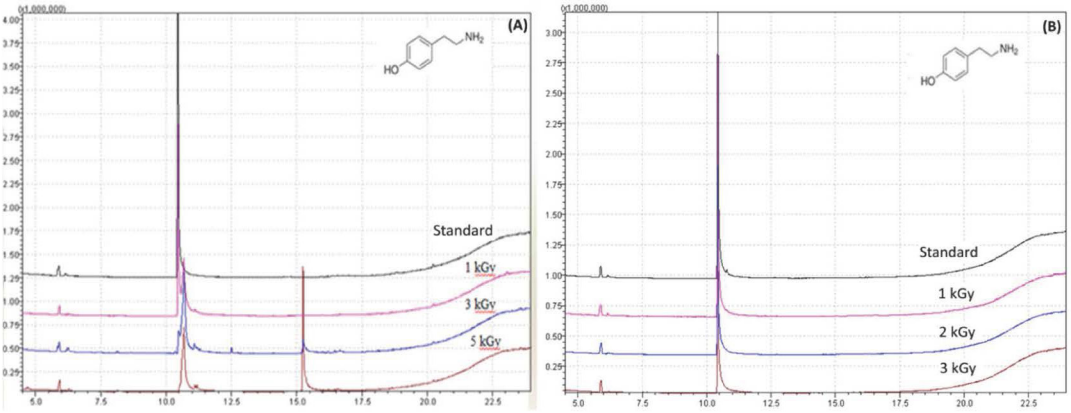


Figure S3. Chromatograms of the methanolic (A) and aqueous (B) solutions of tyramine after irradiation at different doses. Mode: scan; Column: RTX-5MS (5% diphenyl/95% dimethyl polysiloxane); Ramp: 2 min, 80 °C; 10 °C min^{-1}, 80 °C to 280 °C.

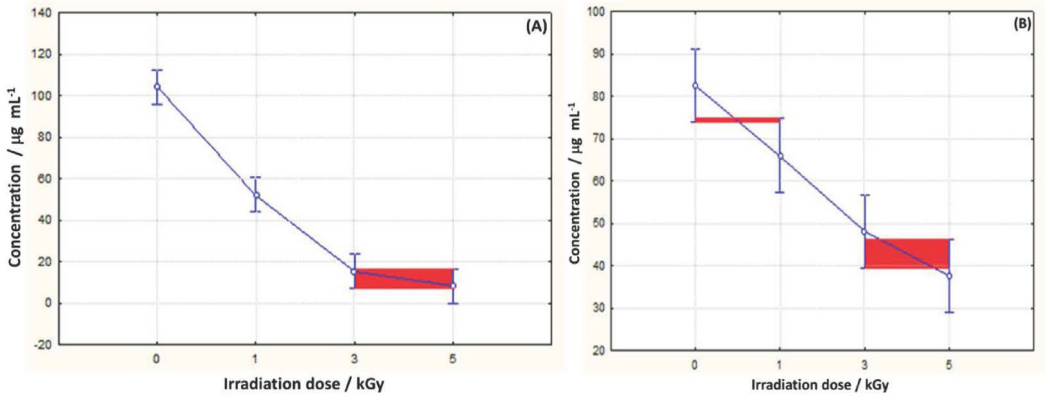
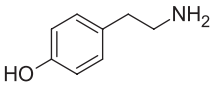
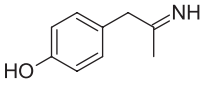
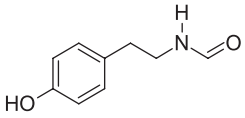
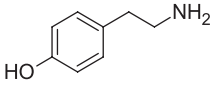


Figure S4. Plot of the tyramine concentration *vs.* irradition dose for the methanolic solutions (A) and the aqueous solutions (B).

Table S1. Main peaks in the chromatograms of the methanolic and aqueous solutions of tyramine irradiated at different doses

Analytes	t_R / min	Average area of the peak at each dose			
		Standard	1 kGy	3 kGy	5 kGy
		Methanolic solutions			
 Tyramine	10.66	13446888	6279797	1209809	225518
 Peak 1	10.81	–	5753838	7722443	5671843
 Peak 2	15.36	–	–	840704	4591475
		Aqueous solutions			
 Tyramine	10.78	10439435	8177844	5690438	4266855