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ANALYSIS OF IRRADIATED BIOGENIC AMINES BY COMPUTATIONAL CHEMISTRY AND SPECTROSCOPY

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ABSTRACT

Biogenic Amines (BA's) are nitrogenous compounds able to cause food poisoning. In this work, we studied the tyramine, one of the most commom BA present in foods by combining experimental measured IR (Infrared) and GC/MS (Gas Chromatograph / Mass Spectrometry) spectra and computational quantum chemistry. Density Functional Theory (DFT) and the Deformed Atoms in Molecules (DMA) method was used to compute the partition the electronic densities in a chemically-intuitive way and electrostatic potentials of molecule to identify the acid and basic sites. Trading pattern was irradiated using a Cs 137 radiator, and each sample was identified by IR and GC/MS. Calculated and experimental IR spectra were compared. We observed that ionizing gamma irradiation was very effective in decreasing the population of standard amine, resulting in fragments that could be rationalized through the quantum chemistry calculations. In particular, we could locate the acid and basic sites of both molecules and identify possible sites of structural weaknesses, which allowed us to propose mechanistic schemes for the breaking of chemical bonds by the irradiation. Moreover, from this work we hope it will be also possible to properly choose the dose of gamma irradiation which should be provided to eliminate each type of contamination.

1. INTRODUCTION

Nitrogen-containing compounds are essential to life, and their main source is atmospheric nitrogen which, by a process known as *nitrogen fixation*, are reduced to ammonia and then be converted to organic nitrogen compound [1]. In particular, amines are basic organic nitrogenous compounds in which one, two or three atoms of hydrogen in ammonia are replaced by alkyl or aryl groups [2]. Like ammonia, amines contain a nitrogen atom with a lone pair of electrons, thus amines are both basic, nucleophilic and their chemistry depends on this property [3].

Amines can be produced in ordinary metabolic processes of living organisms and appear as natural substances in a variety of foods [4-6]. In foods that contain high amounts of proteins, free amino acids and are placed in an environment subject to microbial growth or biochemical activity, the appearance of biogenic amines are expected [2; 4; 5; 7; 8]. Biogenic amines (BA's) are mostly formed by bacterial enzymatic decarboxylation of free amino acids [2; 6; 7], have low molecular weight and aliphatic, aromatic or heterocyclic structure [5; 7-10]. BA's are present in a wide range of food products including fish, meat, dairy, wine, beer, vegetables, fruits, nuts and chocolate [2; 5; 7; 8].

In addition to their biological role as sources of nitrogen and precursors for hormone synthesis, alkaloids, nucleic acids and proteins and amines are also important food aroma components and potential precursors for the formation of carcinogenic N-nitrous compounds [2; 11-13]. Although BA's are needed for several critical physiological processes, consumption of food containing high amounts may produce toxicological effects [2; 4; 7; 14]. BA's may cause disease in men and animals, where toxemias resulting from food ingestion containing biologically active amines have been reported [2; 5; 8; 15; 16]. Determination of BA's concentration and its precursors is important both to establish their toxicity and as indicators of the degree of food freshness, even if the foods do not appear spoiled or organoleptical unacceptable [2; 7; 8; 17-20].

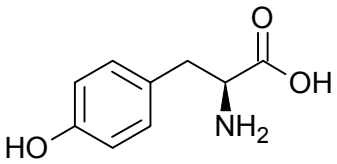
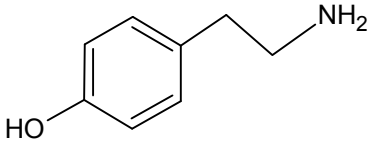
The original concentration of amines in food, that can modify the flavor and the odor substances, and can affect food acceptance, can change as a result of maturation/storage conditions, and because of that should be controlled [5; 7; 9; 12]. The total amount of the different amines formed strongly depends on the nature of the food and the microorganisms present [7]. The meat quality is essential to determine the presence and activity of microbes, so the BA's in food can be controlled by strict use of good hygiene practices in both raw material and manufacturing environments with corresponding inhibition of spoiling microorganisms [5-7; 15; 21]. BA's production has been related to factors such as availability of substrate, pH, salt concentration and temperature [7; 21-24]. The BA's concentration depends on the ripening time, decarboxylase activity of the microflora, in addition to the variation in the quality of the meat, and their presence in food is attributed to protein metabolism (exogenous and endogenous enzymatic action) [5; 10]. The BA's control in foods includes the availability of free amino acids, unfavorable conditions to the microorganisms growth and inhibition of the enzymatic activity [2; 7; 8]. Storage temperature may affect the production of biogenic amines in food, and this is probably the most important method of prevention [7; 15; 17; 21; 25; 26]. As the pH decreases the amine production has been explained as a protective mechanism for bacteria against acidic conditions, but a better understanding of the mechanisms by which biogenic amines are being produced is necessary to prevent their formation [27-30].

In non-fermented foods, the presence of biogenic amines above a certain level is considered as indicative of undesired microbial activity. Therefore, the amine level could be used as an

indicator of microbial spoilage [2; 7; 9; 24; 31]. During the preparation of fermented food one can expect the presence of many kinds of microorganisms, some of them being capable of producing BA's [2; 7; 8; 10]. Shelf life of food can be extended with high temperature treatment because kill the bacterial species, but once that BA's are formed it is difficult to destroy because BA's concentrations were unaffected by cooking and they were not significantly reduced by high temperature treatment [2; 7; 32-34], so is necessary to use the gamma ionization to kill the microorganisms and destroy the biogenic amines because other methos are not efficient to do it.

The presence of high levels of biogenic amines in foods can trigger immediate symptons noted in allergic responses, highlighting the tyramine (e.g. "cheese reaction"), one of the most common foodborne intoxications is caused by it [7; 14; 35-38]. Tyramine (see Table 1) is biologically active amine with important physiological effects in humans, mostly psychoactive or vasoactive. [2; 4; 7; 22; 36; 39-41].

Table 1. Precursor of biogenic amine involved in food poisoning and their pharmacological effects.

Precursors of BA	Biogenic Amine	Pharmacological Effects
 Tyrosine	 Tyramine	✓ Peripheral vasoconstriction ✓ Increases the cardiac output ✓ Causes lacrimation and salivation ✓ Increases respiration ✓ Increases blood sugar level ✓ Releases noradrenaline from the sympathetic nervous system ✓ Causes migraine

Gamma ionising irradiation is used both to eliminate pathogenic microorganisms as reduce biogenic amines, being an important technique to food preservation. The radiation kills the microorganism by destroying the nucleic acid of cells beyond induce the radiolytic degradation of biogenic amines in a dose-dependent manner [15; 42; 43].

Studies showed that irradiation up to 1 kGy was enough to quite reduce the biogenic amines [44]. In fish, irradiation significantly retarded the production of histamine, tyramine, cadaverine and putrescine with 1 to 3 kGy [45].

However, some molecular properties and chemical reactions are very difficult to observe experimentally, and molecular orbital calculations can help us to investigate such situations. The aim of this work is use simple tools for molecular modeling and, together with IR and GC/MS techniques, propose a method of analysis simpler and cheaper, but no less efficient. We want to show that quantum mechanics and molecular modeling can indicate the most easy way to get answers about which energy should be used, where the structure will be broken and what are products that can be expected.

2. MATERIALS AND METHODS

2.1. Samples Preparation and Spectrometric Methods

The solutions were prepared using trading patterns of biogenic amines purchased from Sigma-Aldrich Brazil and methanol HPLC grade, with 100µg/ml concentration. For identification were used gas chromatography associated to a mass detector and infrared spectroscopy. Chromatographic conditions: GC-2010 Gas Chromatograph (Shimadzu Corporation); interface temperature at 200°C; SCAN mode for m/z 15 to 300; helium mobile phase; manual injection; split rate of 1/5; flow rate of 1.00ml/min; heating was linear from 40°C to 300°C, with rate of 10°C/min; chromatographic separation was performed with column RTx-5MS (30m x 0,25mm x 0,25µm). Infrared conditions: IR Prestige 21 Fourier Transform Infrared Spectrophotometer (Shimadzu Corporation); a solution drop was placed in Germanium-coated KBr cell with a pasteur pipette, then it was pressed with another KBr cell to make a film; the cells were placed in the analysis instrument; the data were processed with Solution IR software.

2.2. Irradiation Process

The irradiation process was performed at the Nuclear Defense Section of Brazilian Army, using a cavity type research irradiator, which has a Cs-137 radiation source and maximum dose rate of 1.8 kGy/h, provided with a self-withdraw apparatus controlled by an electric-electronic system and movable shielded doors. The chambers have the dimensions of 138x37x19 cm each, one located above and the other below the source plan. The total weight of its structure is 19 tons. It also shows many safety systems including logical gadgets and fixed settings that can guarantee a perfect functioning, eliminating any possibility of accidental exposure to the radioactive source [46].

2.3. Calculations and Modelling

Calculations were made by Density Functional Theory (DFT) method with B3LYP functional and 6-311+G** basis set [47-49] in Gaussian 03 software [50]. For these calculations we used the software Gaussian 03 in machines with XeonQuad processors, 16 cores and 16GB RAM, with Linux operating system. We considered the overestimation to DFT calculations up to 10% when compared with experimental frequencies (ν_{calc}/ν_{obs}), which is an accepted value in these conditions [51; 52]. Vibrational frequencies were compared through data tables and spectra (experimental data obtained from IR Prestige, and modelling data plotted with Molden 5.0 [53]). For electron densities, density deformations and electrostatic potentials of the optimized geometries was used DAMQT 1.0 [54; 55]. This tool was very useful to indicate acid and basic sites (for nucleophilic or electrophilic attack) and help to propose energetically favorable mechanistics schemes for new structures.

3. RESULTS AND DISCUSSION

For initial molecular structures were used Molden 5.0 [53], then optimized geometry (which indicate a equilibrium structure of molecular system) and frequencies calculations were made with Gaussian 03. In this article we was used DFT Method with the B3LYP functional and the 6-311+G (d,p) basis set. The calculations presented the main frequencies of the molecules, so it is possible to compare them with experimental data from literature [56]. This step is very important to validate de molecular model.

Table 2. Comparison between calculated data and experimental data of tyramine [56]

Tyramine		
ν_{calc} [a]	ν_{obs} [b]	(ν_{calc}/ν_{obs})
270 (167)	-x-	-x-
832 (203)	830 (60)	0,2
1179 (270)	1280 (20)	8,6
1267 (162)	1390 (65)	9,7
1540 (176)	1520 (45)	1,3
1651 (56)	1600 (42)	3,1
2954 (103)	2920 (4)	1,2
3069 (65)	2970 (20)	3,2

a = calculated frequency (relative intensity); b = observed frequency (transmittance %) (cm^{-1})

From samples without irradiation and irradiated with 1, 3 and 5 kGy we obtained infrared and mass spectra. Figure 1 show a weak signal appearing at 2300-2400 cm^{-1} range (see the arrows), which may indicate appearance of new molecules, or new functional groups in substrate, or both, but in all cases the break down of biogenic amines occurred by ionizing gamma radiation.

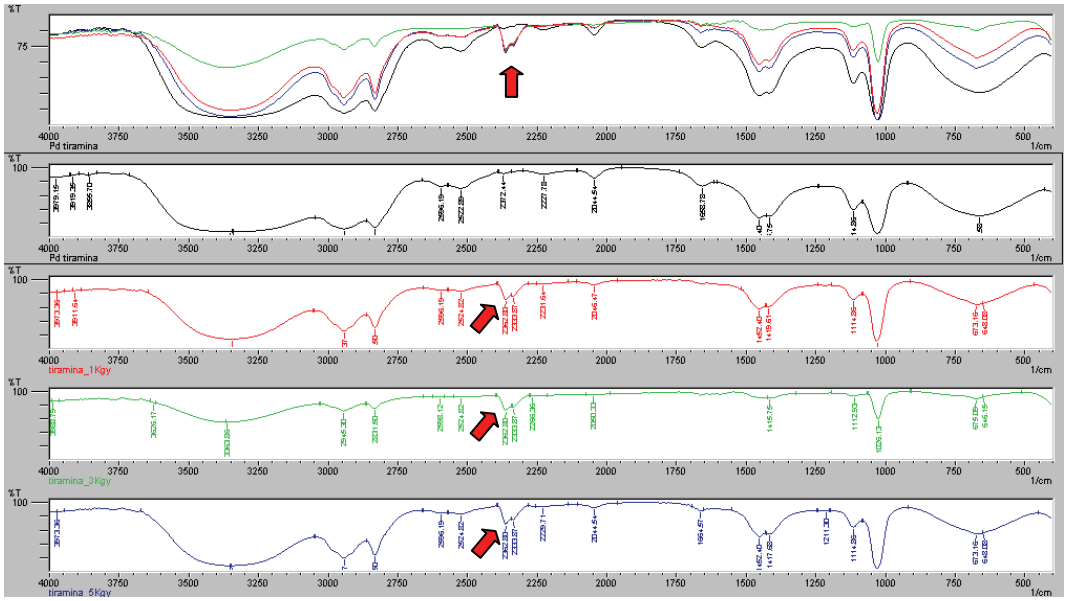


Figure 1. Infrared spectra of tyramine: (i) overlapping; (ii) without irradiation (black); (iii) irradiated with 1kGy (red); (iv) irradiated with 3kGy (green); (v) irradiated with 5kGy (blue). Frequencies in cm^{-1}.

Because the solvent is methanol, many kinds of interferences may be occur in various signals which complicating the infrared analysis. Important signals in several bands may also be

changed but most of them should be under the influence of intermolecular hydrogen bonds made by free hydroxyl in solution (supplied by methanol) [57]. On the other hand, mass spectra do not leave any doubt about the appearance of a fragment, and this is clear when we look the figure 2.

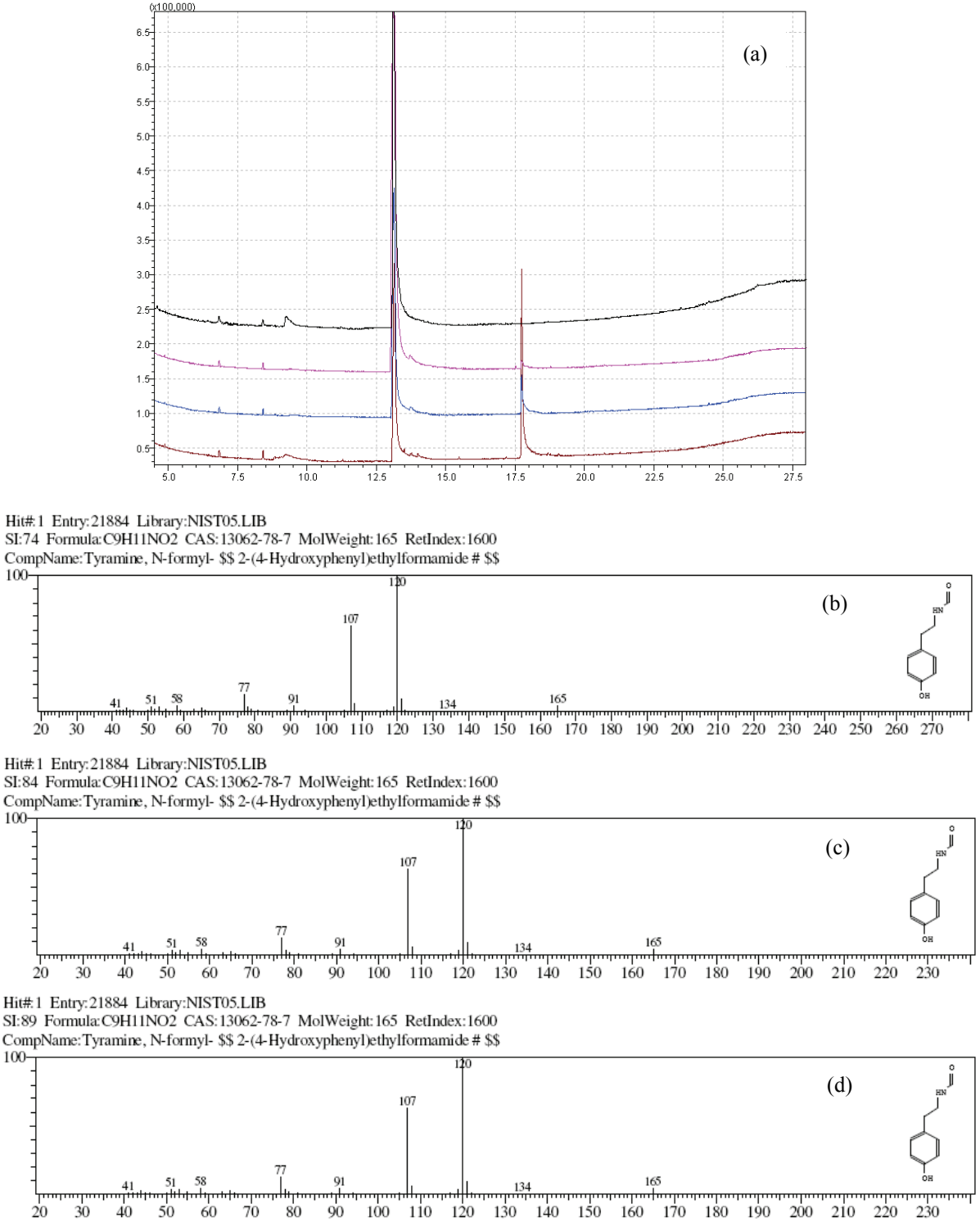


Figure 2. MS analysis of tyramine. (a) Total Ion Chromatogram (TIC) without irradiation (black) and irradiated with 1kGy (pink), 3kGy (blue) and 5kGy (brown); mass spectrum library search for (b) 1 kGy, (c) 3 kGy and (d) 5 kGy.

In Figure 2 appears a retention time at the 17.725 minutes, and we saw that the signal of tyramine was decreasing, thus proving that ionizing gamma radiation is efficient to decrease the concentration. Also note that the similarity signal between mass spectrum library and sample spectra was increased with increasing radiation dosage.

At this moment was used DAMQT 1.0 [54] for analysis of the electron density in molecules, and for this was calculated density deformations and electrostatic potentials. In figure 3 we observe the tyramine structure with highlights to regions near oxygen and nitrogen atoms, which are the most important sites for chemical reactions because there are the largest charge gradients in the molecule. Note that red regions indicate higher concentrations of electrons. Consider for figures 3 and 4: (A) = acid site; (B) = basic site: (S) = strong chemical bond; and (w) = weak chemical bond.

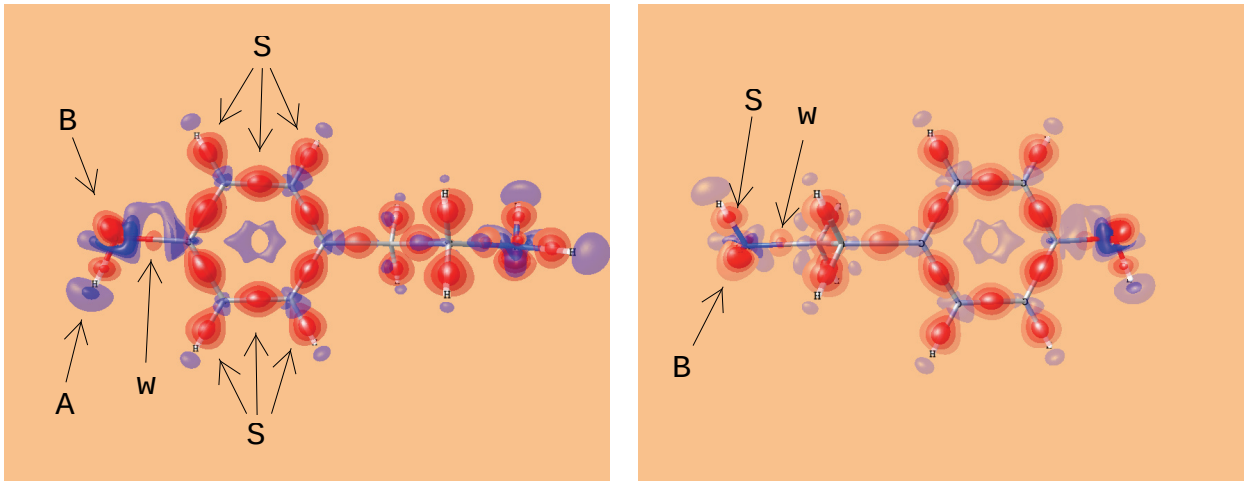


Figure 3. Density deformations of tyramine. Red is positive deformations (charge accumulation) and blue is negative deformations (charge depletion). Contour values: ±0.06, ±0.04 and ±0.02. Units in a.u.

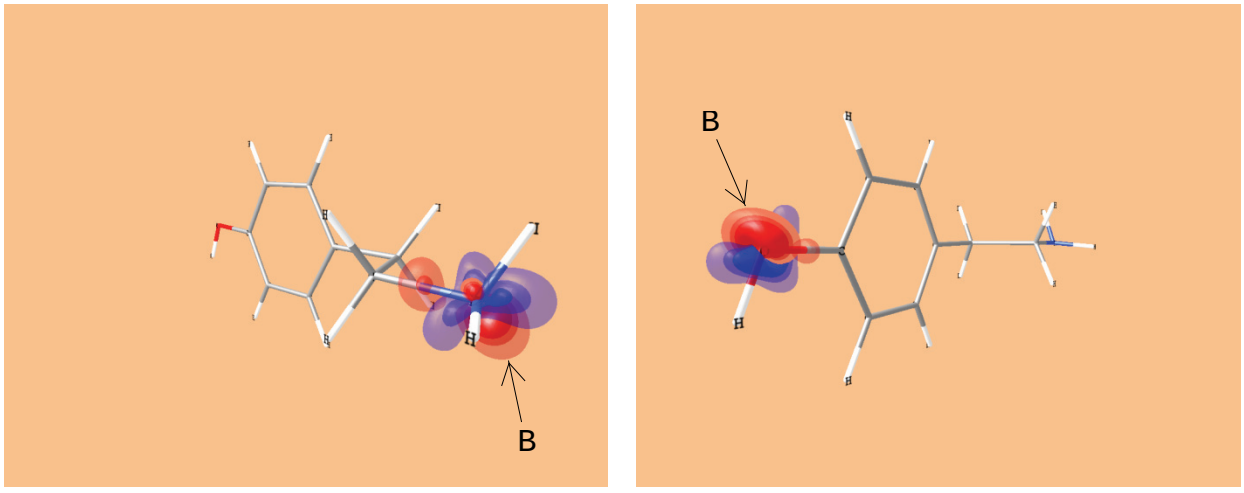


Figure 4. Density deformation of nitrogen (left) and oxygen (right). Contour values: ±0.06, ±0.04 and ±0.02. Units in a.u.

In figure 4 we highlight the density deformations of oxygen and nitrogen atoms without other structure deformations. Note that contour values are larger in oxygen atom, which indicates a

greater content of electron density due to its higher electronegativity. We can identify the chemical bond strengths and can be seen regions of electron accumulation in the bonds too.

Figure 5 shows the tyramine electrostatic potentials and red regions (negative values) indicate higher proton attraction. Observe that negative fields make a protection shield to the carbon skeleton nucleophilic attack (particularly oxigen atom), whereas one side of nitrogen atom is free.

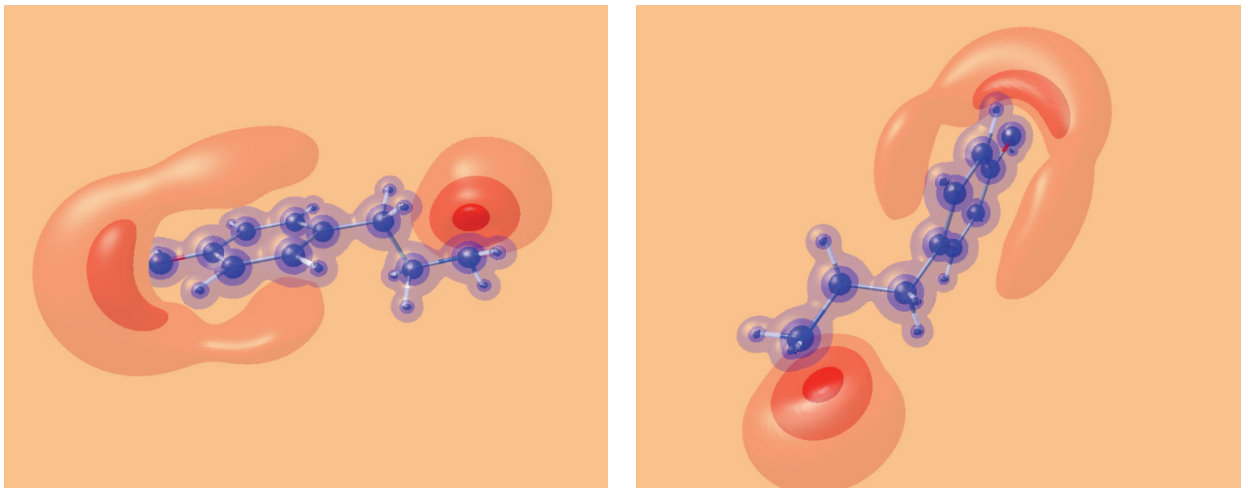


Figure 5. Electrostatic potential of tyramine. Contours: blue: 5, 2 and 0,5; red: -0,10, -0,05 and -0,02. Units in a.u.

Observing the infrared spectra (figures 1 – weak signal appearing at 2300-2400 cm^{-1} range), mass spectra (figures 2 – appearance of a fragment or molecule) and analysis of the electron density (figures 3 to 5) we can propose a mechanistic scheme for tyramine (figure 8). Remember that infrared spectra can suggest (i) C≡C stretching band (weak) of alkyne molecules (occurs in the region of 2260-2100 cm^{-1}), and/or (ii) isonitriles ($R–N^{+}≡C^{-}$), cyanates (R–O–C≡N) and isocyanates (R–N=C=O) show the triple bond or cumulative bond stretch (in the 2280-2000 cm^{-1}) [57].

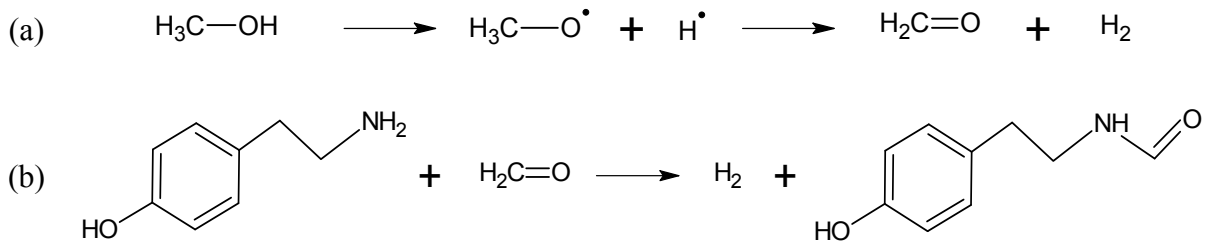


Figure 6: Mechanistic Scheme Proposed: (i) Radiolysis of methanol; (ii) Tyramine.

4. CONCLUSIONS

The mechanistic scheme proposed is fully compatible with spectrometric results, and we confirmed that ionizing gamma irradiation is very effective in decreasing the population of standard amines, giving rise to fragments that could be rationalized through the molecular

modeling calculations. Moreover, from this work we hope to be possible to properly choose the dose which should be provided to eliminate the contamination or avoid biogenic amines formation, and so contribute for food safe. In addition the molecular modeling can also assist in the determination of synthetic routes to point out the strengths and weaknesses of the molecular structure. The authors believe that molecular structures of biogenic amines can help answer many open questions, and molecular modeling becomes a powerful tool when points out possible ways and energetically favorable reactions.

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