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Spectral Characterization by FT-IR of Biogenic Amines

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Abstract. The biogenic amines (BA) are organic bases of low molecular weight biologically active. Foods with favorable conditions for the growth of microorganisms with amino acid decarboxylase may present the formation of BA. Excessive BA oral intake, may induce adverse reactions, such as toxicological effects and changes in blood pressure. BA analysis is important not only because of toxicity, but also to its use as a food quality indicators. Analytical methods commonly used for the determination of BA are the chromatographic, however, it are complex, expensive and it is time consuming during the process; for this reason the development of a technique based on spectroscopy, Fourier transform infrared (FT-IR), that allows an qualitative determination of BA is proposed. Spectral characterization by FT-IR of eight BA was obtained, which could be observed in the characteristic bands of amines, 3300-3500 cm^{-1} N-H stretching, 1500-1650 cm^{-1} bending N-H, 1020-1350 cm^{-1} corresponding to stretching the C-N primary and secondary amines; and the band of 2800-3050 cm^{-1} (vibrational absorptions of C-H bonds) feature of the aliphatic, aromatic and heterocyclic of BA. The FT-IR analysis of each of the AB and AB mixture of 8 helped establish its characteristic spectral response, which established the basis for the qualitative determination of BA by FT-IR spectroscopy.

Keywords: FT-IR, food poisoning, food safety

INTRODUCTION

The BA according to their chemical structures are classified as aliphatic (putrescine, cadaverine, spermine and spermidine), aromatic (tyramine, β-phenylethylamine), heterocyclic (histamine, tryptamine)[1]. The BA

are synthesized and degraded derivative metabolic activity of animals, plants and microorganisms, and play important physiological functions[2]. In the case of microorganisms, the biogenic amines are produced by enzymatic decarboxylation processes amino and trans-amination or amination of aldehydes and ketones. The BA have specific physiological role in the activity of the human brain, the regulation of body temperature and pH of the stomach, gastric acid secretion, immune responses, and cell growth and differentiation etc[3]. In food, with high availability of free amino acids and favorable conditions for the growth of microorganisms with amino acid decarboxylase, may present the formation of BA. Excessive BA oral intake, derived from the consumption of food, may induce adverse toxicological effects such as nausea, headaches, rashes and change in blood pressure reactions. This is especially true in sensitive individuals and those in which BA detoxification is impaired[4]. Food high in protein promotes high levels of BA such as; fish and fish products, dairy products, meat and meat products, fermented vegetables, fermented soy products and alcoholic beverages such as wine and beer. Fish and certain varieties of cheese contain the highest amounts of histamine (up to 1000-2000 mg kg^{-1}), is the food mostly associated with cases of histamine poisoning[5]. Meat and especially cheese have higher concentrations of tyramine. In ripened cheese made from raw milk, tyramine levels can reach more than[6] 1000 mg* kg^{-1}. Histamine is the only BA for which the FDA "Food and Drug Administration" and the European Community have established maximum levels in fish from the family *Scombridae* and *cupleidos* (50 mg / kg and 100 mg / kg respectively), although there is general interest in reducing the presence of all BA in foodstuffs because aliphatic amines to promote intestinal absorption of other amines also enhancing its toxic effects[7].

Determination of biogenic amines in food is important, not only from a toxicological point of view, but also because these compounds can be used as indicators of quality of food. In fresh meat, high levels of putrescine, cadaverine, and tyramine were associated with elevated concentrations of *Pseudomonas spp*. *Enterobacteriaceae* and *lactic acid bacteria*, respectively[8]. The main applications of the analysis of biogenic amines are: quality control of raw materials, intermediates and final products, monitoring of fermentation processes, process control, research and development. Analytical methods for the determination of BA is mainly based on chromatographic methods: thin layer chromatography (TLC), gas chromatography coupled to mass spectrometry (GC-MS), capillary electrophoresis (CE) and high performance liquid chromatography (HPLC) [5]. HPLC is the most commonly used method for the analysis of BA; however these techniques have the disadvantage of consuming time and being costly. This decreases their viability when it is required to analyze large numbers of samples or when products short shelf life. For this reason the development of a technique based on FT-IR allows a qualitative determination of BA proposed.

The aim of this study was to establish the basis for the qualitative determination of BA in food by FT-IR spectroscopy.

METHODS

Histamine (Hist, 97%), Cadaverine (Cad, 97%), spermine (Spm, 97%), spermidine (Spd, 98%) and putrescine (Put, 98%) tryptamine (Tryp, 99%), tyramine (Tyra, 98%), β-phenylethylamine (β-Phe, 98%), biogenic amine standards of high purity were obtained as hydrochloride from Sigma (USA). Eight BA stock solutions were separately prepared, by diluting them in water at a stock concentration of 1mg/ml. The analysis was carried out individually, moreover due to the fact that in the food can be found several types of BA at the same time, an analysis was held using one mixture of 8 BA. The detailed spectral characterization was carried out according to the tables suggested by Coates[9], and Pretsch, *et al*[10], for infrared spectral interpretation.

Results

In Figure 1 it is shown the characteristic FT-IR spectrum of each BA analyzed. Based on the analysis it was observed spectral bands associated to N-H stretching (3300-3500 cm^{-1}), bending N-H (1500-1650 cm^{-1}), stretching of the C-N primary and secondary amines (1000-1350 cm^{-1}); and the band of 2800-3050 cm^{-1} (vibrational absorptions of C-H bonds) related to the structure of aliphatic, aromatic and heterocyclic of BA. The FT-IR analysis of BA did not show any bands corresponding to the symmetric and asymmetric stretching of NH bond in the region of 3300-3500 cm^{-1}, except for the spectrum of the tryptamine, which shows a peak at 3422 cm^{-1}, which also may be associated with "=CH" bond absorption of the aromatic amine structure.

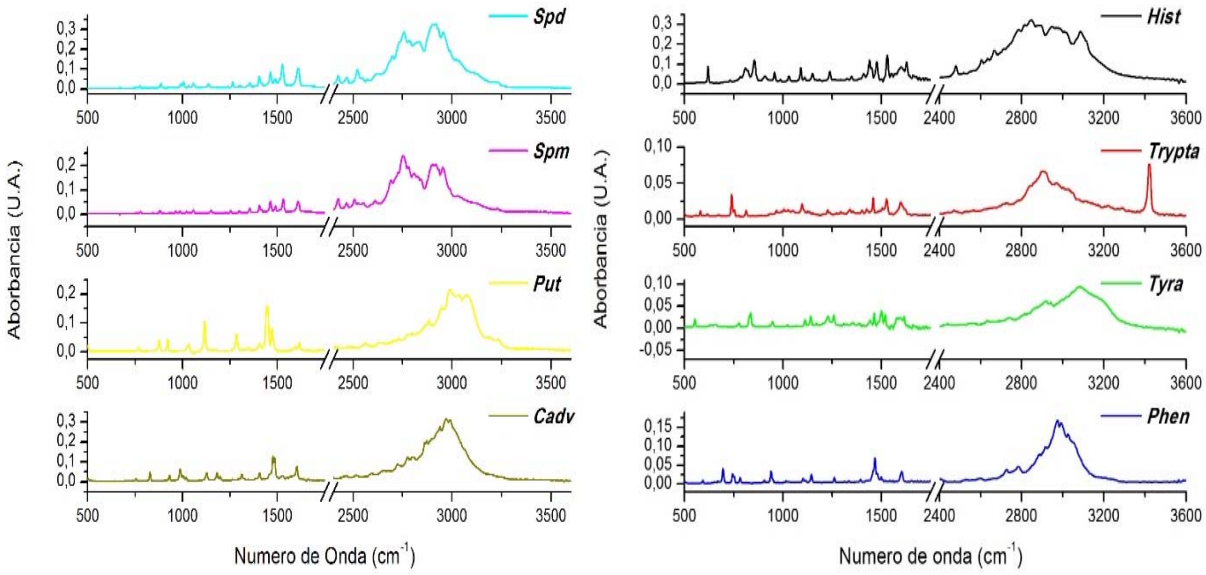

Fig.1: FT-IR spectrum of aliphatic, heterocyclic and aromatic BA.

According to the spectral analysis performed in Figures 1, the absorption peaks characteristic of the bonds contained in the structure of BA were identified. Table 1 shows the results of the spectral characterization.

According to the spectral characterization of each BA, it is possible to discern, their specific chemical structure. Thus diamines can be differentiated by the presence of the wave numbers at 1183, 1312, 1475, 1489, 2971, 2989 cm^{-1} to cadaverine and the wave numbers at 1117, 1446, 2991, 3075 cm^{-1} to putrescine. The polyamines may be discriminated by the presence of the wave numbers at 1254, 1150, 2752 cm^{-1} to spermine and the wave numbers at 1137, 1265, 2918 cm^{-1} to spermidine. The heterocyclic amines can be distinguished by the wave numbers at 1090, 1238, 1476, 2846, 2982, 3087 cm^{-1} to histamine and wave numbers at 1340, 1457, 2905, 3422 cm^{-1} for tryptamine. The aromatic amines can be differentiated by wave numbers at 1228, 1258, 1518, 2921, 3082 cm^{-1} to tyramine and the wave numbers at 1261, 1466, 2974 cm^{-1} for β-phenylethylamine. Figure 2 shows the characteristic spectrum of the mixture of 8 BA. The spectral analysis was performance in the bands between 1400-1700 cm^{-1} and 2600-3600 cm^{-1}. The peaks identified in these bands are shown in Table 2.

Table 1: Spectral characterization for BA.

BA	Type of vibration / Peaks FT-IR (cm^{-1})						
	C-H stretching	C-N stretching			C=C ring skeleton	N-H stretching	CH_2 bending (Twisting)
		primary amine	secondary amine	aromatic primary amine			
Cad	2971, 2989	1183, 1312	-	-	-	-	1475, 1489
Put	2991, 3075	1117	-	-	-	-	1446
Spm	2752	1254	1150	-	-	-	1462
Spd	2918	1265	1137	-	-	-	1462
Hist	2846, 2982, 3087	1090, 1238	-	-	-	-	1476
Tryp	2905	1340	-	-	-	3422	1457
Tyra	2921, 3082	-	-	1228, 1258	1518	-	-
β-Phe	2974	-	-	1261	1466	-	-

Table 2: Peak absorbance for the determination of a mixture of 8 AB

Mix AB	Bond/type of vibration	Peaks FT-IR (cm^{-1})
8AB	CH_2 bending (Twisting), C = C ring skeleton	1468, 1483, 1513
	N-H bending (scissors)	1513, 1610, 1629
	N-H stretching	3372
	C-H stretching	2917, 2853, 2961, 3016, 3037
	= CH aromatic stretch	3016, 3037

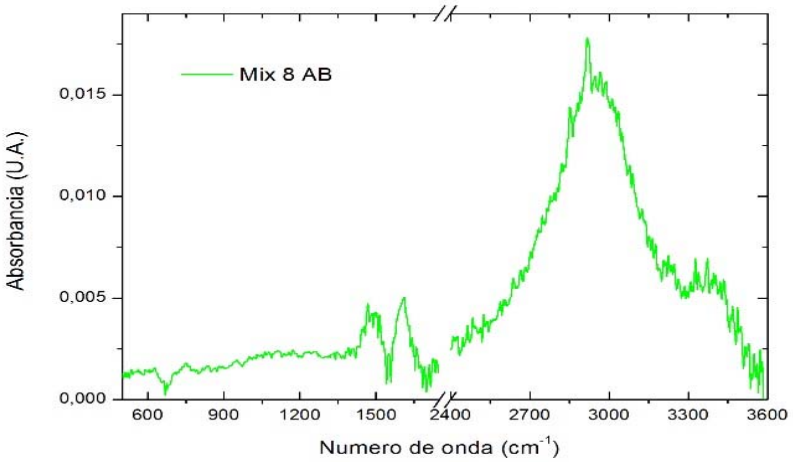

Fig. 2: Peak absorbance for the determination of a mixture of 8 AB.

CONCLUSIONS

Spectral characterization identified in the analysis shows all the peaks in the bands identified as the corresponding to the characteristic vibrations of each of the chemical structures of the biogenic amine. The FT-IR analysis of the mixture of 8 biogenic amine allows to establish its characteristic spectral response. This research established the basis for the qualitative determination of BA by FT-IR.

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