Towards a new rapid methodology to detect fish freshness by measuring the IR spectral behavior of diamine oxidase reaction to different biogenic amines

[1,2]Luis Ramiro Caso Vargas, [1]Raúl J. Delgado Macuil, [1]Valentín López Gayou, [1]Amado Enrique Navarro Frómetra, [1]Orlando Zaca Morán; [1]Centro de Investigación en Biotecnología Aplicada. Tepetitla de Lardizábal, Tlaxcala. 90700. [2]Universidad Tecnológica de Izúcar de Matamoros. Ingeniería en Procesos Alimentarios. I. de Matamoros, Puebla. 74450.
rmrcaso@gmail.com

Key words: FTIR ,biogenic amines diamine oxidase.

Introduction. The presence of biogenic amines (BA) in food is considered to be an indicative of freshness and acceptability but also point to microbial spoilage. That is why their detection in meat, fish, cheese, wines, beer and some other food is important. Traditionally the BA detection protocols use chromatography to detect and quantify them, but it's still a time consuming methodology, thus the possibility to detect histamine, cadaverine, putrescine and spermidine by tracking their respective reaction kinetics using FT-IR has been investigated as a faster alternative.

Methods. Standards of the above mentioned BA were acquired from Sigma. Two different Diamine Oxidases (DAO) were used in the experiments: Sigma's porcine kidney and BioResearch's plant source (0.69U). A 50 mM working solutions of the five BA as free bases were prepared in PBS 0.1M, pH 7. Kinetic reactions were set up in a Bruker Vertex 70 FT-IR Spectrometer in ATR mode at mid-IR region. In order to determine the detection limit multiple kinetics were done varying the concentration of the amines.

Results. An absorbance increase were observed for the four BA at 1641cm^{-1} and 1548cm^{-1} bands using Porcine kidney DAO except for cadaverine that exhibited an absorbance raise at 1635cm^{-1} and 1541cm^{-1} (data not shown). When using plant source DAO all BA showed an absorbance change at 1072cm^{-1} and 989cm^{-1} bands (figures 1 & 2).

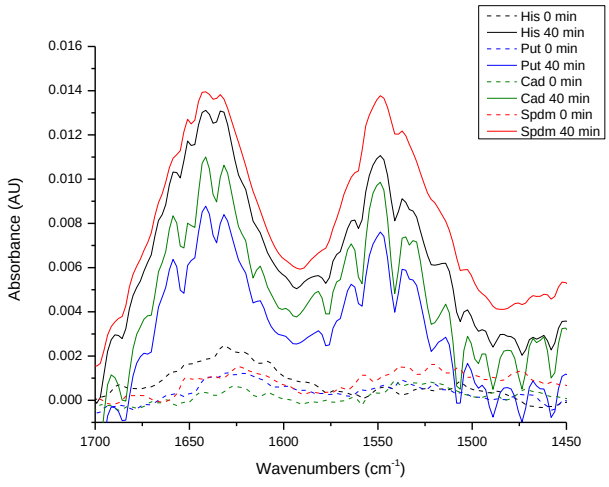

Fig.1 Kinetic reaction of histamine (his), putrescine (put), cadaverine (cad) and spermidine (spdm) using porcine kidney DAO. Dashed lines, time zero, solid lines, after 40 minutes.

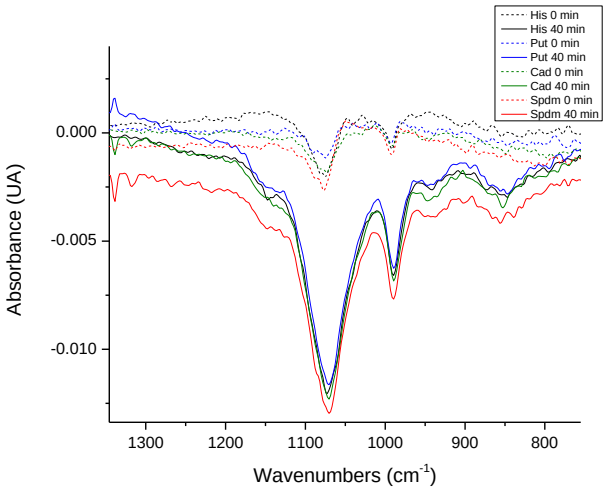

Fig.2 Kinetic reaction of histamine (his), putrescine (put), cadaverine (cad) and spermidine (spdm) using plant source DAO. Dashed lines, time zero, solid lines, after 40 minutes.

From these reactions was determined that the plant source DAO exhibited a higher performance than porcine kidney one. Assays carried on using ethanolic extractions from spoilage tilapia were able to detect BA presence (data not shown). At this moment the limit of detection has been established only for putrescine at 800 ppm *in vitro*.

Conclusions.

The data from this research indicates that with further improvement on sensitivity determining BA with FTIR could be a fast feasible alternative to HPLC or GC/MS to detect and quantify these compounds in fish.

Acknowledgements. Part of this research is being supported by SIP-IPN.

References.

1. Bodmer, S., Imark, C., & Kneubühl, M. (1999). Biogenic amines in foods: histamine and food processing. Inflammation research : official journal of the European Histamine Research Society ... [et al.], 48(6), 296–300.
2. Di Fusco, M., Federico, R., Boffi, A., Macone, A., Favero, G., & Mazzei, F. (2011). Characterization and application of a diamine oxidase from Lathyrus sativus as component of an electrochemical biosensor for the determination of biogenic amines in wine and beer. Analytical and bioanalytical chemistry, 401(2), 707–16.
3. Mureşan, L., Ronda Valera, R., Frébort, I., Popescu, I. C., Csöregi, E., & Nistor, M. (2008). Amine oxidase amperometric biosensor coupled to liquid chromatography for biogenic amines determination. Microchimica Acta, 163(3-4), 219–225.
4. Prodromidis, M. I., & Karayannis, M. I. (2002). Enzyme Based Amperometric Biosensors for Food Analysis. Electroanalysis, 14(4), 241–261