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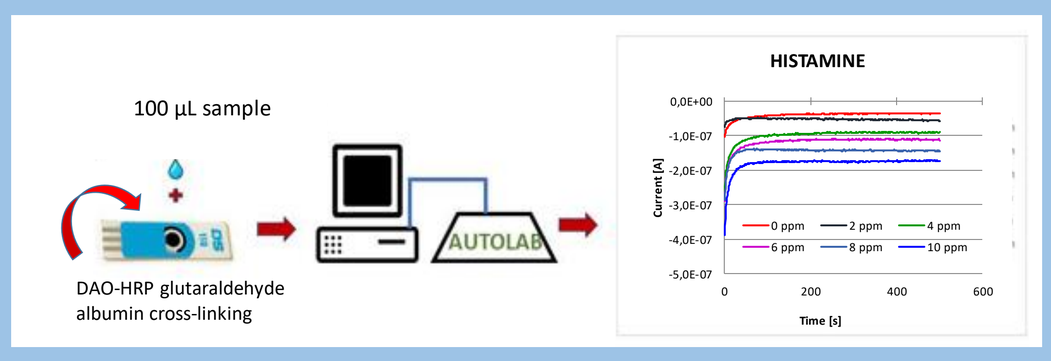
AN ENZIMATIC BIOSENSOR FOR A RAPID DETECTION OF HISTAMINE IN FRESH TUNA

Aim: the aim of our work is to build up biosensors with the capability to detect pathogens and secundary products derived from bacteria methabolism in food matrices.
Here are reported procedures and comparative tests performed through an histamine-elctrochemical biosensor developed in our laboratory.

Histamine intoxication (scombroid poisoning) is the most common cause of human foodborne illness due to the consumption of fish products.
Amperometric biosensors can provide a valid alternative to the official analytical methods. They can detect the hydrogen peroxide formed by the chemical process catalyzed by the enzyme diamine oxidase (DAO). The peroxidase (HRP) catalyzes the electrochemical reduction of H_2O_2 produced by DAO through a direct electron transfer reaction on the electrode surface using a suitable mediator at low operating potential.



- **Samples**: fresh and frozen yellowfin tuna.
- **Microbiological analysis:** enrichment in TSB for the detection of Histamine Producing Bacteria (HPB).
- **Electrochemical analysis:** broth cultures and fish samples positive for HPB, spiked fish samples for recovery test.
- **Comparative test with HPLC-analysis:** samples with different levels of histamine concentration.

RESULTS

- ✓ *Morganella psychrotolerans, Photobacterium phosphoreum* were the bacteria most frequently detected in the enriched samples.
- ✓ All samples that were positive for HPB, gave a signal at electrochemical analysis and in the Table 1 were reported the corrisponding histamine concentration, calculated from the calibration curve.

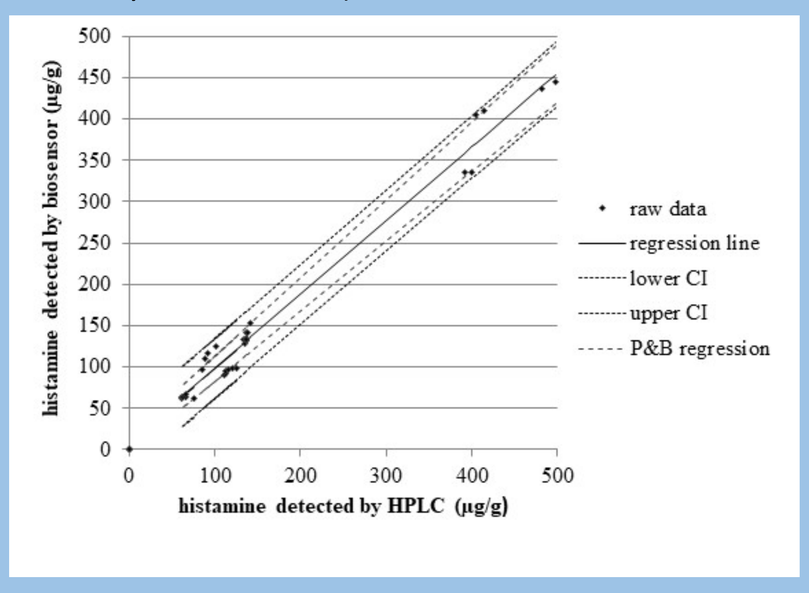
Lot	Histamine			Species
	Culture (µg/ml) [a]	Tuna 7d (µg/g) [b]	Strain (µg/ml) [c]	
1	5,920	24	8,473	M. psychrotolerans
2	5,770	18	6,518	M. psychrotolerans
3	7,880	<LOD	8,506	P. damselae
3	11,046	60	8,212 368 7,312	K. oxytoca H. alvei M. psychrotolerans
5	1,169	20	88 rt; 332	P. phosphoreum
6	2,216	<LOD	412 rt; 250	P. phosphoreum
7	1.451	na	17 rt; 316	P. phosphoreum

Table 1. Histamine-producing bacteria isolated from fresh tuna fillets

[a] histamine produced by sample homogenates enriched in histidine decarboxylase (HD) broth at 20°C for 3 days
[b] histamine in tuna samples stored at <4°C for 7 days
[c] histamine produced by isolated colonies enriched in HD broth

- Figure 1 displays a scatterplot of the histamine levels measured with biosensor and HPLC method in the concentration range 75-498 µg/g (n=6 replicates). The correlation coefficient between the two methods is r = 0.990.

Figure 1. Regression analysis of the two methods (HPLC and enzymatic sensors)



The ability of enzymatic amperometric biosensor to detect histamine, combined with phosphate buffer extraction under high temperature and pressure, allowed the development of a simple, rapid, and relatively inexpensive method to measure histamine in fish and bacterial cultures. The evaluation of testing method carried out in this study shows that it is fit for the purpose of measuring the histamine-producing potential of bacteria and the concentration of histamine in fresh tuna. Histamine biosensors can be a tool that the food operators might use to ensure that the Food Safety Objective is met.

Status: ARTICLE SUBMITTED

Biosensing the histamine-producing potential of bacteria in fresh tuna

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