

Development and validation of an enzymatic reagent for automating histamine analysis in fish



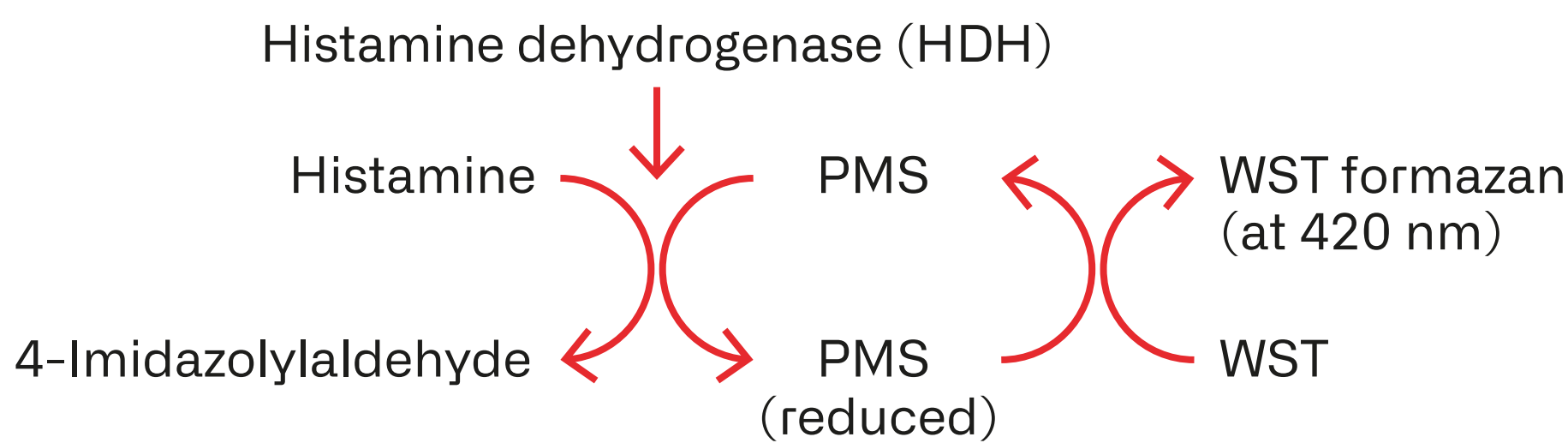
Abstract

The presence of histamine in fish is becoming increasingly important to consumers and producers alike, due to the potential threats of toxicity to humans and consequently health implications. In the scientific field, histamine has the potential to be applied as indicator unsanitary conditions during fish manipulation. Current methods for analysis of histamine are HPLC, ELISA, and fluorimetry which sometimes require expensive and sophisticated instrumentation and as consequence skilled technicians. BioSystems presents a new, simple and rapid enzymatic method determination of histamine in fish that can be automated.

The method is based on the specific reaction of histamine with recombinant histamine dehydrogenase (from *E. Coli*) causing the reduction of a soluble tetrazolium salt to form formazan salt that absorbs at 420 nm. Thus can be measured by visible spectrophotometry and correlate it, through a calibration, with histamine concentration. This new reagent has a long stability, is liquid and ready-to-use, avoiding end-user influence. It has been formulated to be used in any photometer or automated analyzer. Linearity, limit of detection (LoD), limit of quantification (LoQ) , repeatability, within laboratory reproducibility, trueness and recovery were characterized using a BioSystems Y15® automated analyzer and HPLC method and validated following the AOAC guidelines.

Measurement procedure

Histamine in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry:



Sample preparation (fish): Accurately weigh approximately 5 g of homogenized sample and add 25 ml of distilled water. Shake until the sample is homogeneously suspended. Incubate the sample for 20 minutes in a boiling water bath, stirring periodically. Let stand to room temperature and centrifuge for 10 minutes, at least at 2000g. Reagents and calibrators are ready to use and stable until expiry date.

Conclusions

Results confirms that FoodQuality® Histamine Enzymatic (BioSystems) can quantitatively measure histamine in fish samples with similar accuracy and precision than the HPLC method. Furthermore, this test can be automated in a analyzer (the kit is adapted for Y15® or Y25® analyzers from BioSystems) or can be used in a photometer with a simple sample treatment making histamine analysis more accurate, fast and easy. The small volumes used also allows an affordable analysis and better waste management. This new reagent has a long stability, is liquid and ready-to-use, avoiding end-user influence. It has been formulated to be used in any photometer or automated analyzer. Linearity, limit of detection (LoD), limit of quantification (LoQ), repeatability, within laboratory reproducibility, trueness and recovery were characterized using a BioSystems Y15® automated analyzer according to AOAC validation protocols.



Bibliography

- Food and Agriculture Organization of the United Nations/World Health Organization (2013) Public Health Risks of Histamine and other Biogenic Amines from Fish and Fishery Products. Meeting Report
- Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, Method 937.07
- Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, Appendix K
- Tsuneo S. , et al. (2005) Anal. Biochem. 346, 320–326

Product Performance

Limits of detection / Limits of quantification

	Detectability (ppm)		RSDcalculated (%)			Recovery	
	LoD	LoQ	10 ppm	50 ppm	100 ppm	10 ppm	100 ppm
Raw Tuna	0.29	0.90	7.8	3.2	3.9	100%	101%
Water-Packed Tuna	4.28	13.0	4.8	3.0	2.0	108%	100%
Oil-Packed Tuna	4.58	14.0	0.9	1.3	1.5	100%	101%
Raw sardines	7.01	21.0	4.9	1.8	2.6	100%	101%
Oil-Packed sardines	5.77	17.0	3.8	1.9	1.0	98%	94%
Semi-Preserved Anchovy Fillets	2.85	8.50	4.3	3.4	1.5	98%	98%

Reference Materials

Organizer	Matrix	Reference	Assigned value (ppm)	BioSystems (ppm)
FAPAS	Canned Fish	TET040RM	16.6 +- 0.9	16.4
FAPAS	Canned Fish	27176	216 (186-247)	204

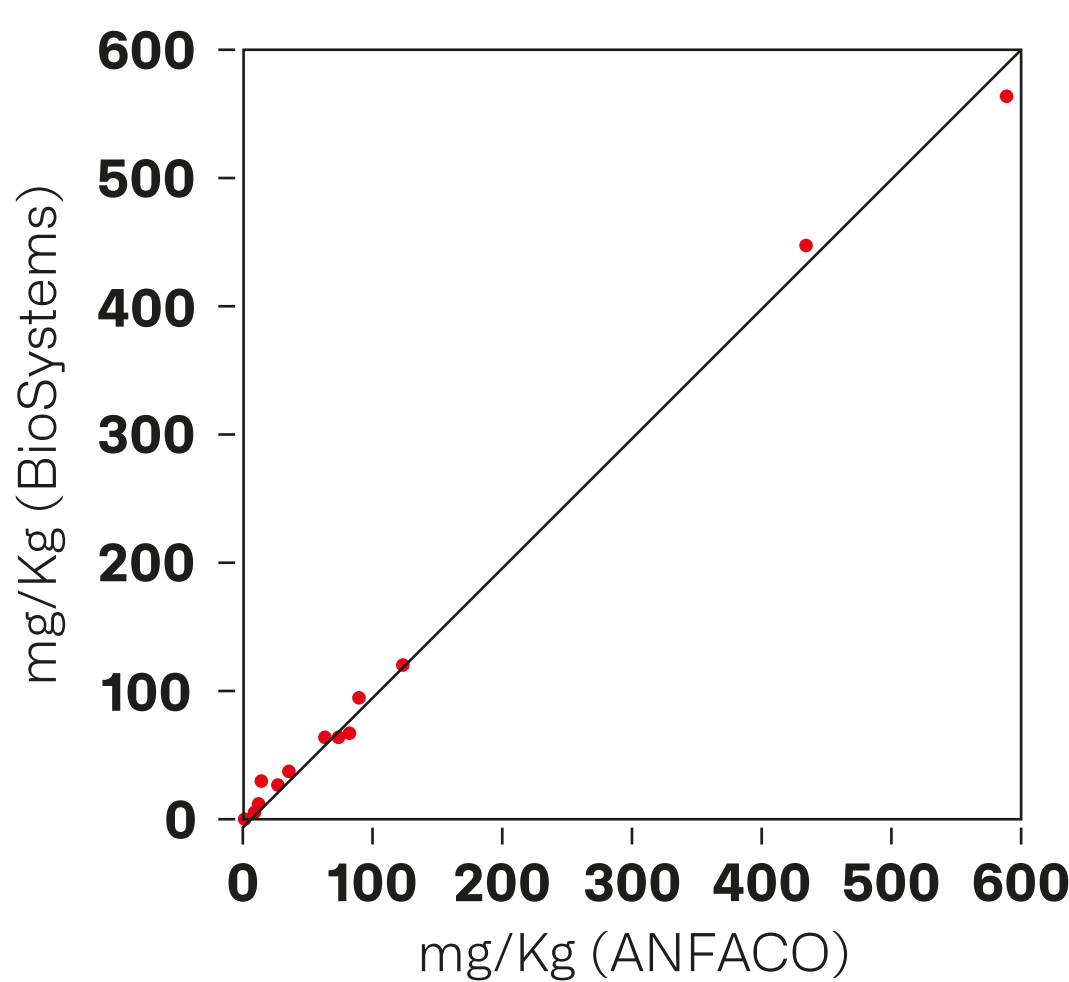
Ring Trial

Ring Trial	Matrix	Reference	Z-score	Ok?
FAPAS	Canned Fish	27189	0.6	Yes
FAPAS	Canned Fish	27253	1.4	Yes
FAPAS	Canned Fish	27243	1.0	Yes

Trueness

Range of measurement = 10 to 200 mg/Kg
Type of method: Quantitative (mg/Kg = ppm)
Reference method used: HPLC-UV accredited by ENAC according to UNE-EN ISO/IEC 17025
BioSystems method: Enzymatic determination

	HPLC	BioSystems
Raw Mackerel	34	39
	61	64
Raw Sardine	10	12
	89	97
Pickled anchovies	592	567
	437	450
Anchovies <i>paté</i>	4	4
Semi-preserved anchovies	4	4
Raw Tuna	0	0
	74	65
	1.1	1.1
Water-canned Tuna	5.5	5.5
Oil-canned Tuna	3	2
	8	8
	122	121
	6.6	6.5
Oil-canned Sardine	7	7
	27	28
Oil-canned Mackerel	0	1
	81	69



Best-fit values	
Slope	0.9813 ± 0.01074
Y-int. when X = 0.0	0.7169 ± 1.833
95% CI	
Slope	0.9587 to 1.004
Y-int. when X = 0.0	-3.135 to 4.569
Goodness of fit	
r	0.9978
Data	
Number of X values	20
Equation	
y = 0.9813x + 0.7169	