

# A BIOCHEMICAL TEST FOR THE DISTINCTION OF FRESH FISH FROM FROZEN AND THAWED FISH

SUDHIR K. CHHATBAR AND N. K. VELANKAR\*

Central Institute of Fisheries Education, Bombay-58

It is observed that the freezing and thawing of fish leads to increase in the total activity of aspartate aminotransferase (AAT) in tissue fluid due to the release of the bound form of mitochondrial enzyme. Electrophoresis of the tissue fluid of fresh unfrozen fish shows only a single fast-moving band of AAT in the anodic region whereas frozen and thawed fish shows an additional slow-moving band corresponding to mitochondrial AAT in the cathodic region. The method can be adopted to distinguish fresh fish from frozen and thawed fish.

## INTRODUCTION

Like mammalian tissues, the presence of sarcoplasmic and mitochondrial isoenzymes of aspartate aminotransferase (AAT) (L-aspartate: 2 oxoglutarate aminotransferase EC 2.6.1.1.) in the fish muscle has been confirmed (Chhatbar and Velankar, unpublished). It is well known that mitochondrial enzymes are more easily extracted after repeated freezing and thawing of the tissue. The present communication reports the influence of freezing and thawing on the extractability and electrophoretic pattern of AAT in the tissue fluid of fresh fish (unfrozen) and thawed fish respectively. Observations made on four fish species, viz. *Scombe-*

*romorus guttatus*, *Pampus argenteus*, *Scoliodon* sp. and *Labio rohita* are presented in this paper.

## MATERIALS AND METHODS

Pieces of muscle of the fish were centrifuged at 20,000 x g. for fifteen minutes. The centrifuged tissue fluid thus obtained was used for the examination of the enzyme activity. AAT activity in the tissue fluid was assayed spectrophotometrically using coupled enzyme reaction system described by Bergmeyer (1963). One unit of enzyme activity is defined as that quantity which converts 1 $\mu$  mole substrate per minutue at 30°C. Electrophoresis of tissue fluid was carried out

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\*Present address: Dept. of Industrial Fisheries, University of Cochin, Ernakulam, Cochin - 682 016

TABLE I  
INFLUENCE OF FREEZING & THAWING ON AAT ACTIVITY  
IN THE TISSUE FLUID OF FISH (U/ml.)

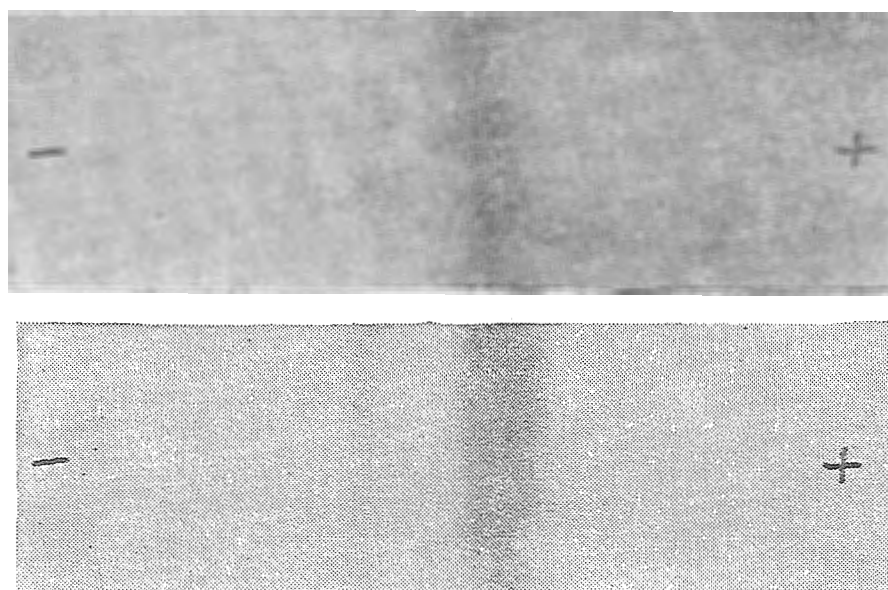
Species	(Fresh	Frozen and thawed
1. <i>Scomberomorus guttatus</i>	2.25	5.50
2. <i>Pampus argenteus</i>	2.55	4.35
3. <i>Scolidon</i> sp.	12.09	17.25
4. <i>Labeo rohita</i>	6.40	8.47

using cellulose acetate membrane (Beckmann microzone system R-101, barbital buffer pH 8.6, 150 V. for 1.5 hr.). The enzyme becomes visible as pink band after spraying the membrane with Fast Violet B salt and same reagents as used for assay. After spraying with assay reagents, the enzyme could also be located as a dark band against fluorescent back-

ground when observed under ultra violet lamp with a 365 nm. filter.

#### RESULTS AND DISCUSSION

The AAT activity of the four representative species of fish, in fresh and, frozen and thawed conditions respectively, is shown in Table I. It is evident from the results that freezing and thawing



↑ AATm    ↑ AATs  
Electrophorograms showing the single band of AATs in the tissue fluid of unfrozen fish (upper) and two bands of AATs & AATm in the tissue fluid of frozen and thawed fish (lower)

Plate - I

results in a significant increase in the AAT activity in the tissue fluid.

The tissue fluid from fresh fish shows only a single fast-moving band in the anodic region on the electrophorogram. In the tissue fluid of frozen and thawed fish an additional slow-moving band in the cathodic region becomes visible (plate 1). Investigations have shown that the anodic band corresponds to the sarcoplasmic isoenzyme of AAT. It was also noted that ice storage of fish prior to freezing does not alter the pattern.

In addition to the above four fish species, similar observations have been made on other fish such as horse mackerel (*Megalopsys cordyla*), dhoma (*Otolithes* spp.), cat fish (*Arius* spp.), flat fish (*Platycephalus* sp.) and mrigal (*Chirrhina mrigala*). The possibility of applying the test for distinguishing fresh fish from frozen and thawed fish, which is often

used as 'fresh fish' in the case of several commercially important food fishes is clearly seen from the above results. It may be mentioned that earlier Kormendy and Hamm (1967) have suggested the use of a similar procedure as a test for pork and beef.

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#### REFERENCES

- Bergmeyer, H. U. 1963. *Methods of enzymatic analysis*; Academic Press, New York and London.
- Kormendy, L. and R. Hamm. 1967. *Proceedings of 8th Hungarian meeting for Biochemistry*, Szeged, p. 45.