

## ABSTRACT

HYPOXANTHINE - ITS QUANTITATION AND RELATIVE IMPORTANCE  
AS A FISH FRESHNESS INDICATOR.

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One of the more frequently used indicators for fish freshness determination is that of hypoxanthine. In this thesis a flow injection method (FIA) for hypoxanthine determination in fish muscle tissue (carite - *Scomberomorus brasiliensis*) is developed using xanthine oxidase immobilized onto 2-fluoro-1-methylpyridium activated Fractogel support system. This FIA system was optimized with respect to its flow rate ( $1.0 \text{ mL min}^{-1}$ ), bioreactor length (1 cm), and sample loop size ( $150 \mu\text{L}$ ). Under these optimized conditions a detection limit of  $4.4 \mu\text{mol L}^{-1}$  for hypoxanthine determination can be achieved. This compares overall favourably with the standard HPLC (high pressure liquid chromatography) reference method for hypoxanthine determination.

In the process of optimization of this FIA system, the flow kinetics of the immobilized xanthine oxidase was also investigated. The kinetic data obtained was analysed by the Lilly, Hornby, and Crook model, and showed that this immobilized enzyme has a  $K'_{m(\text{app})}$  of  $33 \mu\text{mol L}^{-1}$ , a  $C'_{\text{max}}$  of  $200 \mu\text{mol min}^{-1}$ , a pH optimum in the range 7.5 - 8.5, and a temperature optimum range of  $35\text{-}40^{\circ}\text{C}$ . The system also showed excellent storage stability- retaining 60 % of its activity after 9 months when stored in phosphate buffer (pH 8.0) at  $4^{\circ}\text{C}$ .

The hypoxanthine content from various carite samples were compared statistically (multivariate analysis) with other chemical (total fat, volatile acids (TVA), and bases (TVB)), physical (textural attributes) and microbiological indicators of fish freshness as well as with sensory evaluation.

One-way ANOVA tests ( $\alpha=0.05$ ) suggested a significant difference in the values of all the chemical, physical, and microbiological indicators obtained from carite samples from the different locations/storage conditions.

Cluster analysis, using the agglomerative hierarchical clustering technique, showed that clustering probably occurred in four groups. This

analysis also showed good correlation and partitioning when compared to the sensory evaluation.

In factor analysis, using the principal component analysis, five factors were extracted. Factor loadings relate factor 1 (tastiness) with hypoxanthine concentration, TVA and TVB; factors 2, 3, and 5 (texture) with the textural attributes, and factor 4 (rancidity) with the fat content. Rotation of the reference axes showed that factors 1, 2, and 5 remained unchanged, while factors 3 and 4 were interchanged.

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