

# FRESHNESS TESTING PAPER

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## INTRODUCTION

Measuring K value by means of ion exchange chromatography and spectrophotometry is tedious and cumbersome. The Freshness Testing Paper (FTP)\* technique, aims at practicality and suitability for use in the field. The principles involved utilise enzyme actions to convert inosine (HxR) and hypoxanthine (Hx) to uric acid, which changes the colour of the dye present in the paper.

This method should preferably be used after it had been calibrated against the ion exchange chromatographic method. The enzymes present in fishes may vary from species to species, and calibration should be conducted for each species. For very crude estimation, no calibration is required.

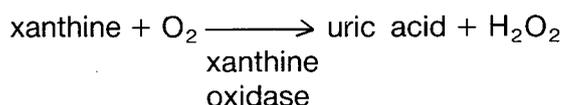
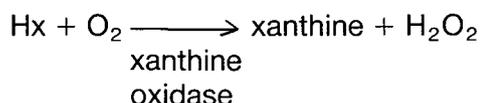
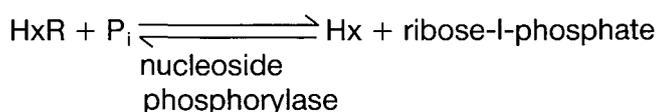
Since the FTP uses enzymes, storage of the test paper at low temperature is essential (preferably at  $-60^{\circ}\text{C}$ ) to ensure the functionality of the enzymes. This is one of the major disadvantage of this technique.

## PRINCIPLE OF FRESHNESS TESTING PAPER

This technique uses enzymatic degradation and the subsequent colour conversion of a redox dye to indicate "freshness"

The K value (%) is defined as 
$$\frac{\text{HxR} + \text{Hx}}{\text{ATP} + \text{ADP} + \text{IMP} + \text{AMP} + \text{HxR} + \text{Hx}} \times 100$$

In the FTP, the enzymes nucleoside phosphorylase and xanthine oxidase are embedded in the test paper. On application of the sample extract, the following reactions occur.



The uric acid formed changes the colour of the redox dye present in the FTP. The colour intensity is proportional to the content of HxR + Hx.

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\* The FTP Test Kit is patented and sold by Kankyo Bunseki Centre K.K. Tokyo.

## **I PROCEDURE**

1. Take 0.5 g minced fish meat and add 0.5 g treated sand. Grind well in a small mortar.
2. Add 1.5 ml of FB\* solution. Grind well. Add another 3.5 ml FB solution and grind well.
3. Dip FTP into homogenised solution. Remove and blot off excess solutions on filter paper. Keep the FTP in a plastic bag (transparent; and keep at room temperature for 10-15 min. (Standardise the time for each species).
4. Compare the colour (red to purple) of FTP with the colour chart provided. Read the corresponding K value.

## **STANDARDISE FTP TO K VALUE BY ION-EXCHANGE CHROMATOGRAPHY**

1. Samples of the species under study of varying freshness is required.
2. Meat samples were individually prepared. A portion of the meat is used in the FTP, while the corresponding portions are subjected to conventional K value analysis.
3. The colour intensity and the corresponding K value are correlated. Care should be taken to standardise the time of reading of the coloured strips of FTP.

## **PRECAUTIONS**

1. All the materials supplied with the FTP kit are easily degraded, and should be stored frozen ( $-60^{\circ}\text{C}$  preferable) and in the dark. Anaerobic conditions will prolong the shelf life.
2. Check the expiry date before use. Expired products will give unreliable results.
3. The resulting colour is unstable in light, and will eventually fade. Readings should be conducted immediately after the full colour had developed.

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\* The procedure for FB solution preparation as stated in the test kit.