DETERMINATION OF K-VALUE BY THE FRESHNESS METER

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INTRODUCTION

The Freshness Meter (Model KV-101, Oriental Co., Tokyo) comprises a water bath incubator chamber fitted with an oxygen electrode, a digital display and a chart recorder. The system uses a series of enzyme reaction steps to break down the ATP and related compounds which are reflected as oxygen consumed during the reactions. The data are presented both in digital read-out and graphically. The ratio of the decrease in oxygen during the various reactions are used to calculate the K-value of the test sample.

The main principles involved in the use of this equipment are presented in Figures 1 and 2.

REAGENTS

A: Sample extraction reagents

a) 10% trichloroacetic acid (TCA)

b) 10 N potassium hydroxide (KOH)

c) Methyl red indicator

B: Reaction reagents

The following reagents are purchased from the equipment supplier or can be mixed in the laboratory.

a) Reagent B
Buffer solution

b) Reagent P is a mixture of alkaline phosphatase and adenosine deaminase.

c) Reagent E_o is a mixture of nucleotide phosphorylase and xanthine oxidase.

PROCEDURE

A Sample Preparation

Take approximately 2 g fish meat. Add 5 ml of 10% TCA and homogenise. Filter, using a coarse filter paper. Add one to two drops of methyl red indicator, and add 10N KOH to neutralise. This neutralised extract is labelled sample S_1.

B Initial Preparation

1) Set the water incubation bath to 37°C and allow the temperature of the reaction chamber to reach 37°C.

2) Place sufficient Reagent B into a bottle and place in water incubation bath. Put in airstone and apply air bubbles to saturate Reagent B with oxygen. Allow Reagent B to equilibrate at 37°C.

3) Zero the digital readout and graph recorder by switching the "FUNC" switch to "ZERO". Use the "ZERO" switch to adjust display to ± 0.1. Similarly, use the "POSITION" switch on the chart recorder to adjust the pen position to zero on the chart paper. Return the "FUNC" switch to "MEAS".

C Preliminary reaction (See Fig. 2)

1) Fill a test tube with 50 ul of Reagent P and 50 ul of extract solution S_1. Place the test tube in the water bath incubator. Allow reaction to occur for 15 minutes. This reactant solution is labelled S_2.

D Main measurement procedure (See Fig. 2)

1) Add approximately 1.2 ml of preheated Reagent B into the reaction cell.

2) Cap the reaction cell tightly. Ensure no air bubbles are trapped inside the reaction cell.

3) Switch on chart recorder and stirrer.

4) Add accurately 20 ul of sample S_1. Wait for one minute.

5) Add accurately 15 ul of Reagent E_0.

6) Take measurement D_1.

7) Add exactly 40 ul of sample S_2.

8) Take measurement D_2.

9) Computation of results. (See Fig. 1)

E Preparing reaction cell for next sample

1) Remove cap and drain out waste reactants.

2) Add distilled water into cell and flush several times.

REFERENCE

Oriental Electric Co. Ltd. (1987) : Manual of Freshness Tester (Model KV-101)

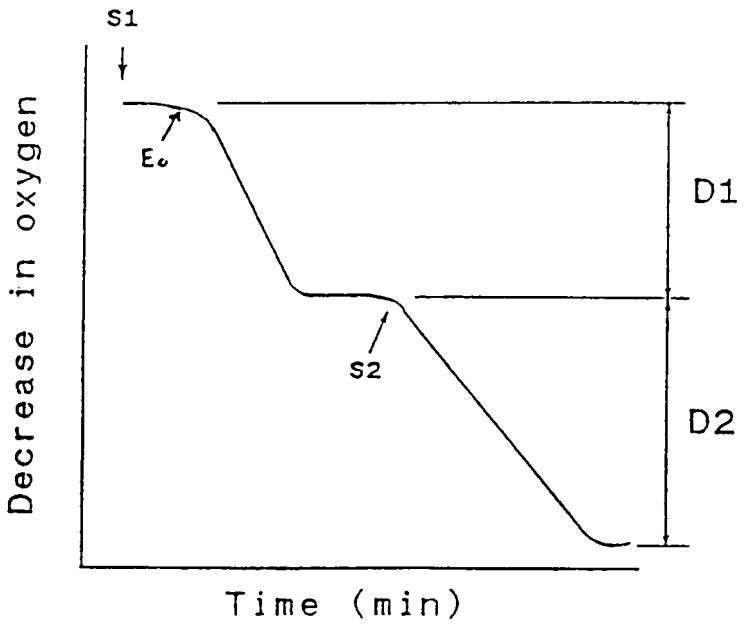

Fig. 1 Calculation of K-Value

$$\text{K-value (\%)} = \frac{D1}{D2} \times 100$$

STEP 1

Original fish extract S_1 is reacted with
Reagent, P (alkaline phosphatase + adenosine deaminase)

ATP + ADP + AMP + IMP + HxR + Hx

Converted to HxR

Resulting solution S_2 contains $\sum$ HxR + Hx

STEP 2

Original fish extract S_1 is reacted with
Reagent Eo (nucleotide phosphorylase + xanthine oxidase)

ATP + ADP + AMP + IMP + HxR + Hx

Converted to uric acid
Oxygen consumed : D_1

STEP 3

Resulting solution from STEP 1, reacted with Eo

$\sum$ HxR + Hx

Converted to uric acid
Oxygen consumed : D_2

STEP 4

$$\text{K value (\%)} = \frac{D_1}{D_2} \times 100$$

Fig. 2 Major Steps in using the Freshness Meter