DETERMINATION OF K VALUE

NG C. S.

INTRODUCTION

The K value is an index to measure the enzymatic freshness of fish and squids. Immediately after death, ATP (adenosine triphosphate) and related compounds are broken down by endogenous enzymes. A typical schematic breakdown can be represented as:-

$$ATP \longrightarrow ADP \longrightarrow AMP \longrightarrow IMP \longrightarrow HxR \longrightarrow Hx$$

ADP = adenosine diphosphate
AMP = adenosine monophosphate
IMP = inosine monophosphate
HxR = inosine or hypoxanthine riboside
Hx = hypoxanthine

The K value is defined as

$$K\% = \frac{[HxR] + [Hx]}{[ATP] + [ADP] + [AMP] + [IMP] + [HxR] + [Hx]} \times 100$$

Ideally, the K value should be measured before exogenous enzymatic activities such as bacterial enzymes begin. In applying K value, care should be exercised to ensure that it is reliable. For example, the K value of a processed fillet may be higher as water soluble components such as IMP may have been washed away. Sampling the unexposed meat will prevent such an error. Skins and dark muscles of fish should be excluded during sampling. Guanine found in the skin will be eluted with the hypoxanthine fraction while dark muscles have a high inosine content.

The present method cannot be directly used for measuring the K value of squids. In the squid, the AMP breaks down directly to HxR. Separation of AMP and HxR is more difficult compared to separation of IMP and HxR. A modified method as proposed by Uchiyama (1984) should be adopted.

PRINCIPLE OF ION EXCHANGE CHROMATOGRAPHY

Ion exchange chromatography distinguishes one component in a mixture from another on the basis of the number of charges of appropriate sign available on each molecule for interaction with the ion exchanger under the conditions imposed. Molecular size is an important factor and the distribution of charges also plays a role.

Ion exchangers can be classified into two categories:-

(i) those that bear positive charges and are called anion exchangers because they interact with anions.

(ii) those that bear negative charges and are called cation exchangers because they interact with cations.

In the present method, an anion exchanger (Cl^- form) is used. Uchiyama et. al (1972) had reported that using authentic mixtures of ATP and its related compounds charged with Dowex

1-X4 column, the elution of HxR and Hx takes place in the region of pH 6.0 and 0.1M NaCl, while nucleotides such as ATP, ADP, AMP and IMP are eluted at a acidity of less than pH3, and within the range of up to 0.15M NaCl.

I PREPARATION OF SAMPLE

One gram of ordinary muscle from fish is sufficient. Care should be taken to exclude red muscle, fibrous tissues and skin. Sample treatment procedure is illustrated in Scheme 2.

II REAGENTS

A. FOR SAMPLE PREPARATION

All reagents listed here should be kept at 5°C until used.

a) 10% perchloric acid (PCA): Dissolve 10 g of PCA (60-70%, $HClO_4$) in 90 ml of distilled water.

b) 5% PCA: Dissolve 10 g of PCA in 190 ml of distilled water.

c) Neutralized PCA: Neutralize 100 ml of 5% PCA to pH 6.4 with 10N-KOH using pH meter, then filter precipitates ($KClO_4$) through filter paper after cooling the neutralized PCA at 5°C.

d) 10N-KOH: Dissolve 56 g of potassium hydroxide (KOH) in distilled water and make up to 100 ml.

e) 1N-KOH: Dissolve 5.6 g of KOH in distilled water and make up to 100 ml.

B. FOR ION-EXCHANGE CHROMATOGRAPHY

a) 0.5M NH_4OH solution: Dilute 4 ml of 25% NH_4OH with 96 ml of distilled water.

b) Solution A = 0.001N HCl: Dilute 1 ml of 1N HCl standard solution to 1000 ml with distilled water.

c) Solution B = 0.01N HCl containing 0.6M NaCl: Dissolve 35.07 g of NaCl in distilled water, then mix this NaCl solution with 10 ml of 1N HCl standard solution and make up to 1000 ml with distilled water finally.

d) Anion exchange resin: AG (R) 1-X4, 400 mesh Cl (chloride)-form (Bio-Rad Co.).

C. PREPARATION OF ION-EXCHANGE RESIN (SCHEME 1)

a) Acetone

b) 0.1N NaOH

c) 0.1N HCl

III APPARATUS

Chromatography System

Figs. 1 and 2 show two systems for simplified method estimation of K-value.

Column

As shown in Fig 3, use the column (inner ∅ 6 mm) with coarse glass filter at the bottom part. The height of resin is around 50 mm.

IV PROCEDURE

A. PREPARATION OF ION-EXCHANGE RESIN

See Scheme 1.

B. PREPARATION OF SAMPLE EXTRACT

See Scheme 2.

C. CHROMATOGRAPHY (Also see Scheme 3)

1. Take 2 ml of neutralized muscle extract in a test tube.
2. Adjust pH to 9.4 by using pH test paper, with a few drops of 0.5M NH_4OH.
3. Apply it onto the column.
4. Wash the inside wall of the column with a few ml of distilled water.
5. In system 1 (Fig 1), onto a column attach a siphon tube which is set in a beaker containing 20 ml of distilled water. In system 2 (Fig 2), attach a separating funnel instead of a siphon onto the column and pour 20 ml of distilled water into the separating funnel.
6. Wash out unabsorbed ultraviolet-absorbing-compounds with distilled water from the column.
7. Pour 45 ml of solution A into the beaker or the separating funnel to elute hypoxanthine riboside (HxR) and hypoxanthine (Hx).
8. Collect the eluate in a 50 ml volumetric flask. Maintain the flow rate at 1-1.5 ml/min.
9. After all the solution A had passed into the resin, run 45 ml of solution B into the column to elute ATP, ADP, AMP and IMP.
10. Collect the eluate in another 50 ml volumetric flask.
11. Make up the eluates to 50 ml with solutions A and B, respectively.
12. Measure the absorbance of the two eluates at 250 nm.

V CALCULATION

$$K(\%) = \frac{E250nm\ A}{E250n\ A + E250nm\ B} \times 100$$

where E250nm A: [OD at 250nm of the solution A-eluate] −[OD at 250nm of the soln A];

E250nm B: [OD at 250nm of the solution B-eluate] − [OD at 250nm of the soln B].

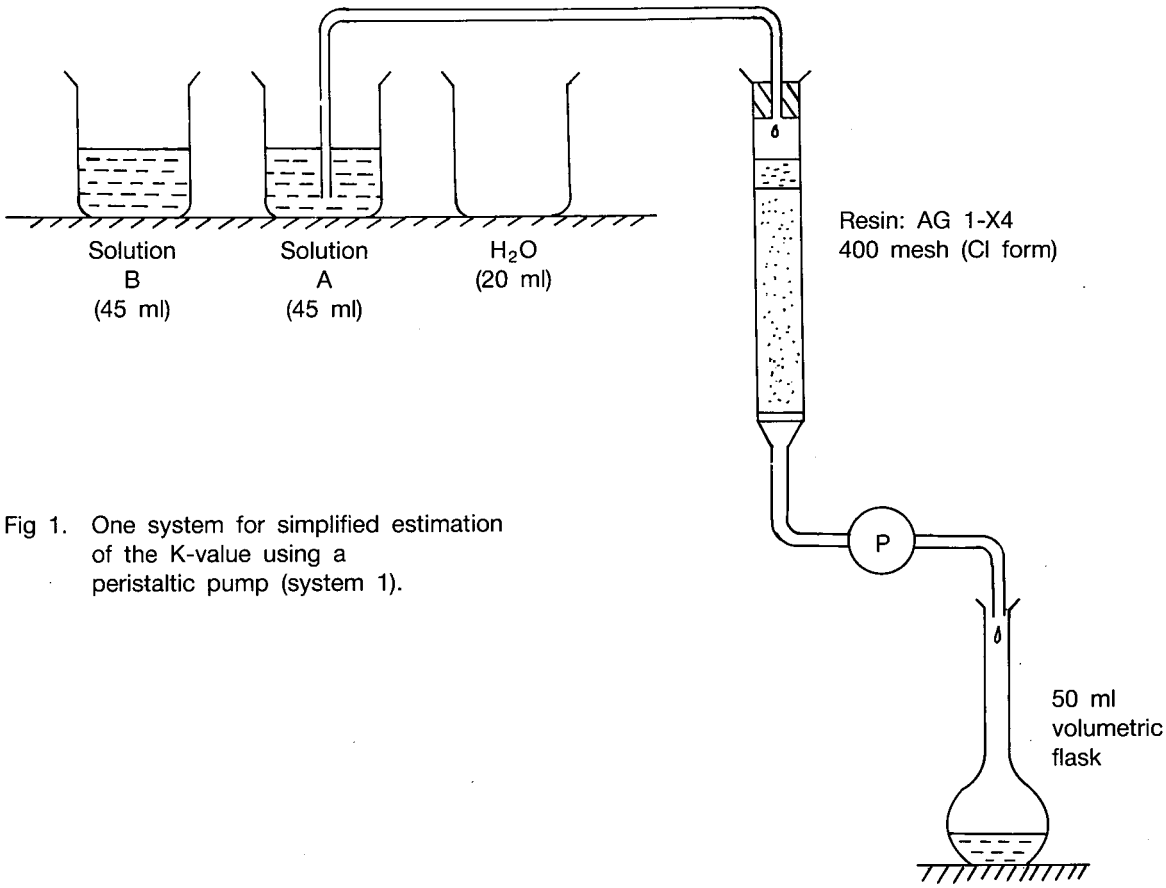


Fig 1. One system for simplified estimation of the K-value using a peristaltic pump (system 1).

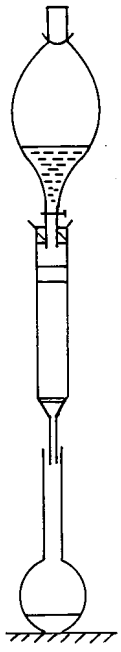


Fig 2. Another system for the estimation of K-value using a separating funnel (system 2).

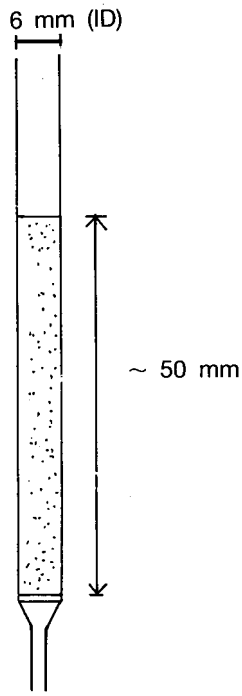
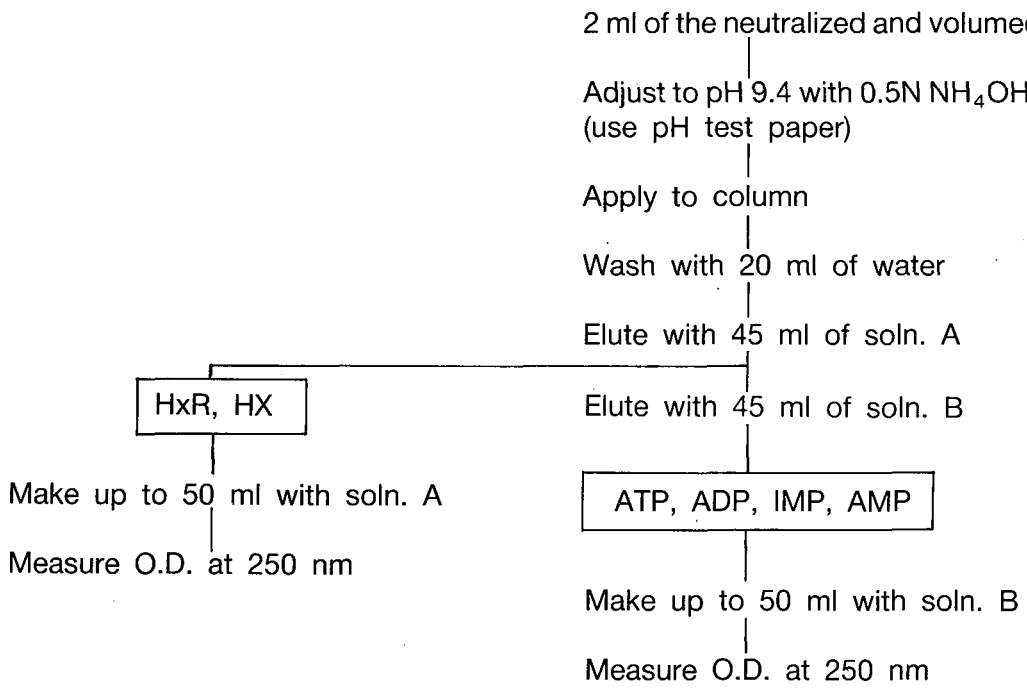


Fig 3. Column

SCHEME 3. SIMPLIFIED FRACTIONATION METHOD FOR K-VALUE



REFERENCES

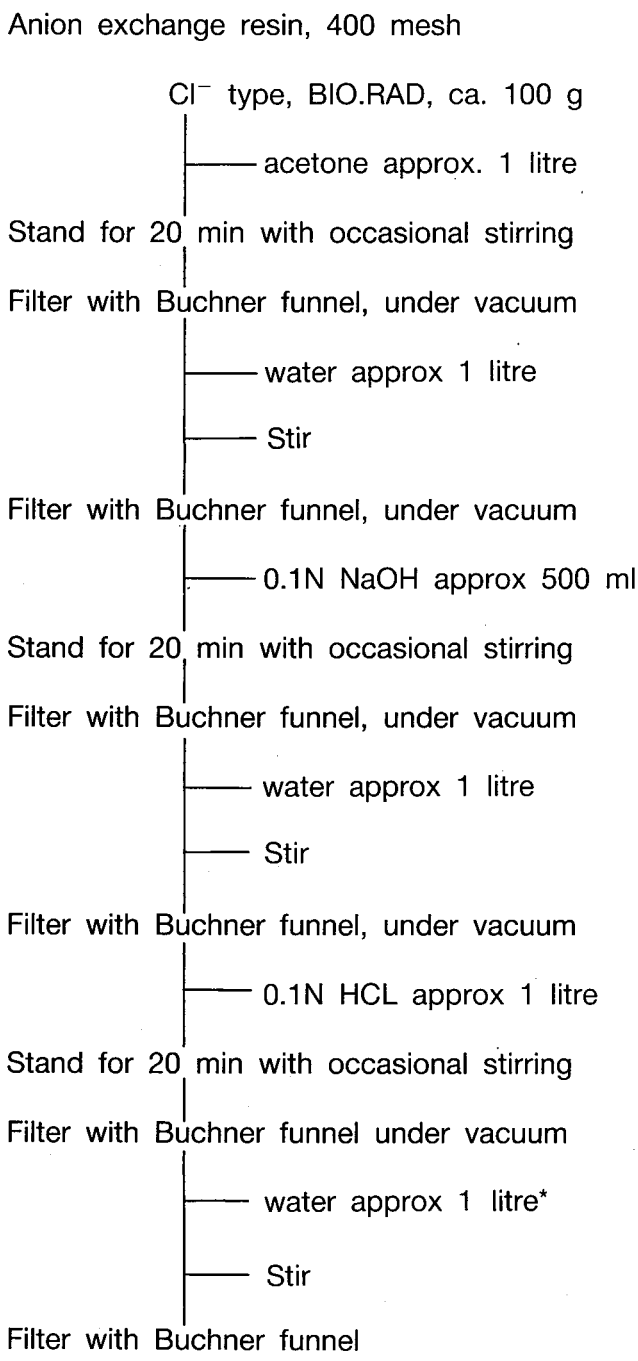
Ehira S., H. Uchiyama, F. Uda and H. Matsumiya. (1970).
A rapid method for determination of acid soluble nucleotides in fish muscle by concave gradient elution. Bull. Jap. Soc. Sci. Fish. 36:391-496.

Uchiyama H., Ehira S., and Kato N. (1972).
Analytical methods for estimating freshness of fish. In Okada et al (Eds). Utilization of marine products. Publ: Overseas Technical Cooperation Agency Govt. of Japan.

Uchiyama H. (1978).
Analytical method for estimating freshness of fish. Training Dept. SEAFDEC: 1-9.

Uchiyama H. and Kakuda K. (1984).
A simple and rapid method for measuring K value, a fish freshness index. Bull. Jap. Soc. Sci. Fish. 50(2):263-267.

SCHEME 1. PREPARATION OF ION-EXCHANGE RESIN



* Repeat washing with distilled water until filtrate (water) is neutral. Activated resin is stored at 5°C under water.

SCHEME 2. PREPARATION OF FISH MUSCLE EXTRACT