Short Paper

High Performance Liquid Chromatographic Determination of K Value as an Index of Freshness of Fish^{*1}

In 1959 SAITO *et al.*¹⁾ suggested that the freshness of raw or frozen fish can be well estimated by the inosine and hypoxanthine content, i.e. K value. This was confirmed by UCHIYAMA *et al.*²⁾ who investigated the relationship between the K value and the freshness of various kinds of fishes judged by sensory evaluation. Since then the K value has been widely used as a measure of freshness of fish. However, the procedure for determination of K value have been time-consuming and troublesome.

This paper deals with a rapid method for determination of K value of fish using high performance liquid chromatography (HPLC). The HPLC used is a JASCO FLC-A700 equipped with UV spectrophotometer UVIDE-100 (250 nm) as detector and Shimadzu Chromatopack C-R1A as an integrator. The column used is a stainless steel tubing (1'×1'/4 i.d.) packed with μ -Bondapak C₁₈. A mixture of 0.05 M (NH₄)₂HPO₄-EtOH (95:5) was used as an eluent and the flow rate of the eluent was controlled at 1.5 ml/min.

To test the separation of ATP and its related compounds, a standard mixture of ATP, ADP, AMP, IMP, HxR, and Hx was subjected to HPLC under the above mentioned conditions. As shown in Fig. 1, three peaks were obtained with retention times between about 3.5 and 5 min. The first of these represented an unresolved mixture of ATP, ADP, AMP, and IMP, and the second and the third represented Hx and HxR, respectively. Therefore, the K value can be easily calculated from the area percentages of these peaks printed with Chromatopack C-R1A.

The accuracy of this HPLC was examined by analyzing the K value of standard mixtures of ATP, HxR, and Hx which were prepared to give the K values ranging from about 10 to 90%. The results obtained are shown in Table 1. The K values obtained by the HPLC well coincided with the calculated ones.

Then the HPLC was applied to estimating the freshness of fish, i.e. the K values of carp *Cyprinus carpio* and rainbow trout *Salmo gairdneri irideus* were determined at appropriate intervals during storage at 5°C. The perchloric acid extract was prepared in the usual manner. The results obtained are shown in Table 2. In both fishes, the K values continued to

Table 1. K value of standard mixture of ATP, HxR, and Hx estimated by HPLC

Calculated*	Analyzed
10.8%	10.6%
21.4	21.6
35.3	34.2
42.1	40.2
52.1	50.1
61.4	59.2
70.5	68.1
80.7	79.0
90.2	88.7

* Calculated from the optical densities of ATP, HxR, and Hx solutions determined at 250 nm before mixing.

Fig. 1. High performance liquid chromatogram of a standard mixture of ATP and its related compounds.

increase throughout the storage period of six days. These results clearly indicate that the K value serves as a useful index of freshness of fish and that the HPLC is a quick and simple method for determination of K value of fish.

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Table 2. Changes in K values and VBN contents of carp and rainbow trout during storage at 5°C

		Storage day				
		0	1	2	4	6
Carp	K value (%)	16.7	24.2	—	65.8	83.0
	VBN (mg/100 g)	5.33	12.1	—	8.77	11.8
Rainbow trout	K value (%)	3.20	45.1	57.8	79.7	83.4
	VBN (mg/100 g)	6.73	11.5	12.0	12.1	13.6

(Received June 29, 1981)

^{*1} 高速液体クロマトグラフィーによる K 値の測定法.

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