High Speed Separation of ATP and its Degradation Products By Extreme Liquid Chromatography (X-LC®) and its Application to Analysis of Fish Freshness

Introduction

ATP(adenosine triphosphate) in fish meet decomposes in a following process:

ATP → ADP(adenosine diphosphate) → AMP(adenosine monophosphate) → Inosinic acid → Inosine → Hypoxanthine

A parameter “K value” indicates freshness of fish meat and is defined as below.

K value(%) = [(Inosine + Hypoxanthine) / (ATP + ADP + AMP + Inosine + Hypoxanthine)] x 100

When K value is below 20 %, fish meat can be used for Sashimi (raw use) and 20% through 60% for cooked and processed use. We applied X-LC to an analysis of K value and evaluated its performance in comparison with results of conventional HPLC.

Experimental

The chromatography system utilized in this experiment consists of JASCO X-LC series: two of 3185PU solvent delivery pump, 3080DG degasser, 3180MX high pressure mixing device, 3067CO column oven, 3070UV UV/Vis detector, 3159AS autosampler, and ChromNAV chromatography data system. Separation column used was a X-PressPak AQ-C18-W (3.0 mm ID x 50 mmL, 2 µm). K value was obtained by a program calculation function in ChromNAV.

Pretreatment of fish meat was conducted in the following procedure.

(1) Add 0.4 M perchloric acid solution (20 mL) to fish meat (2.5 g) to homogenize.
(2) Centrifuge it at 3500 rpm for 10 min.
(3) Add 2 M potassium carbonate solution (1 mL) to the supernatant (5 mL) and centrifuge it at 3500 rpm for 5 min.
(4) Filter supernatant obtained at (3) with 0.45 µm membrane filter.

Results and Discussion

Figure 1 shows an X-LC chromatogram of standard mixture. X-LC succeeded in providing an analysis time eight times shorter than conventional HPLC. Figure 2 shows an X-LC chromatogram of tuna preserved for two days. As shown in this figure, six kinds of components provide a good separation without interfering with unknown components. K value of this sample was 8%. Figure 3 shows relationship between preservation days and K values of tuna and sea bream.

Keywords: X-LC, K value, ATP, ADP, AMP, Inosinic acid, Inosine, Hypoxanthine

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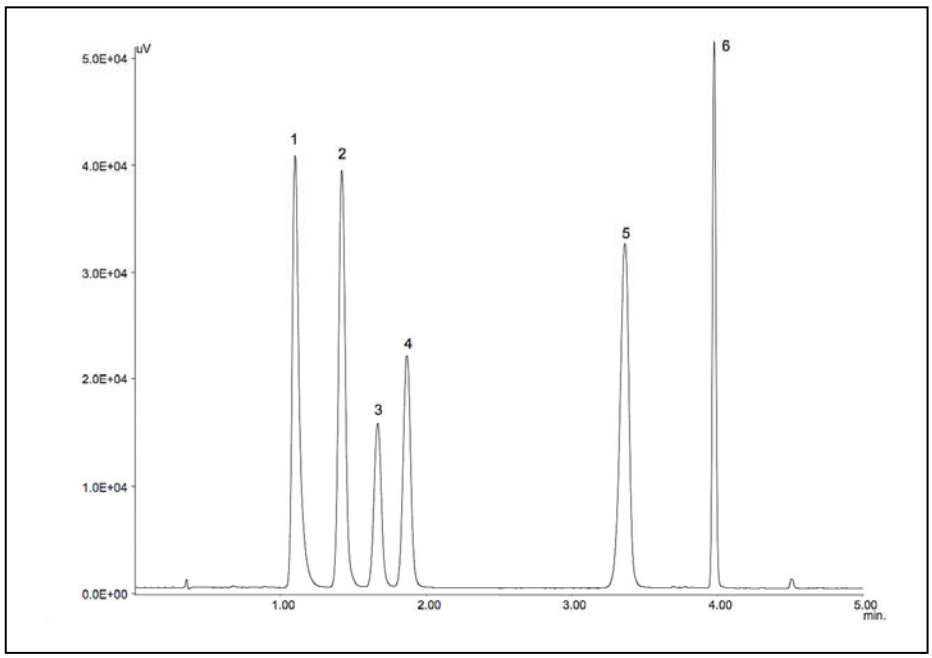

Figure 1 X-LC chromatogram of a standard mixture
Peaks: 1=adenosine triphosphate (ATP), 2=adenosine diphosphaete (ADP), 3=inosinic acid (IMP), 4=hypoxanthine (Hypo), 5=adenosine monophosphate(AMP), 6=inosine (Ino) Chromatographic conditions: Column=X-Presspak AQ-C18-W (3.0 mm ID x 50 mmL, 2.0 µm) Mobile phase: A=100mM phosphate buffer (pH 4.2), B=100 mM phosphate buffer (pH 4.2)/ acetonitrile (50/50) Gradient profile: 0min, A/B (100/0); 2.2 min, A/B (50/50); 7 min, A/B (50/50); 8.2 min, A/B (0/100); 8.3 min, A/B(100/0)

Flow rate: 0.6 mL/min Column temperature: 30°C Detection wavelength: 260 nm Injection volume: 1 µL (1nmol each)

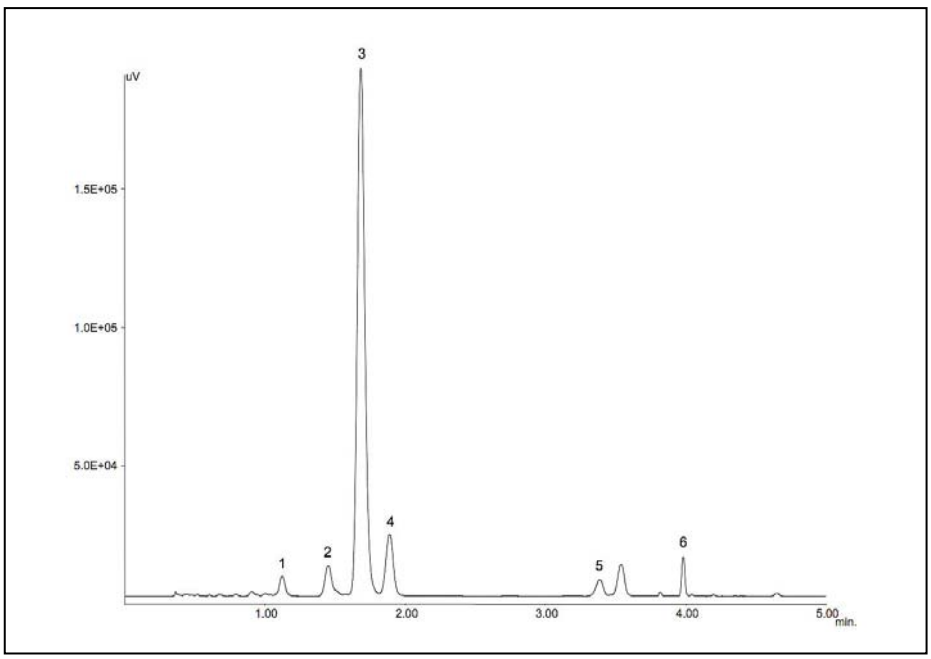

Figure 2 *X-LC* chromatogram of tuna sample
The pretreatment is describe in *Experimental.* The other conditions are the same as in Figure 1 caption.

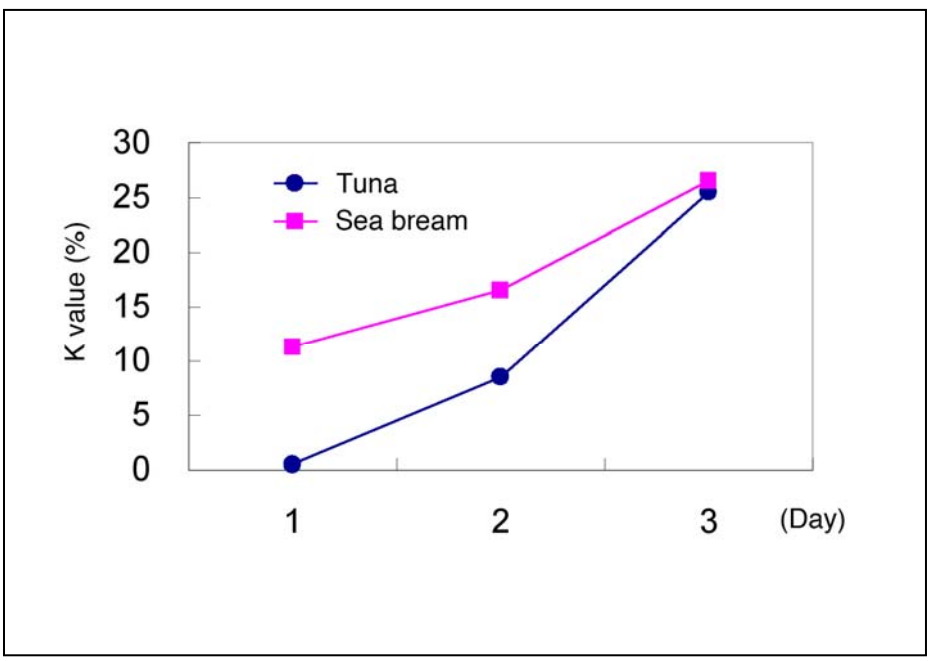

Figure 3 Relationship between preservation days and K values.