Application Note

High Speed Separation of ATP and its Degradation Products By Extreme Liquid Chromatography (UHPLC) 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.

Jasco UHPLC System

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Experimental

The chromatography system utilized in this experiment consists of JASCO X- Cseries: 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 usedwas 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 Mperchloric 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

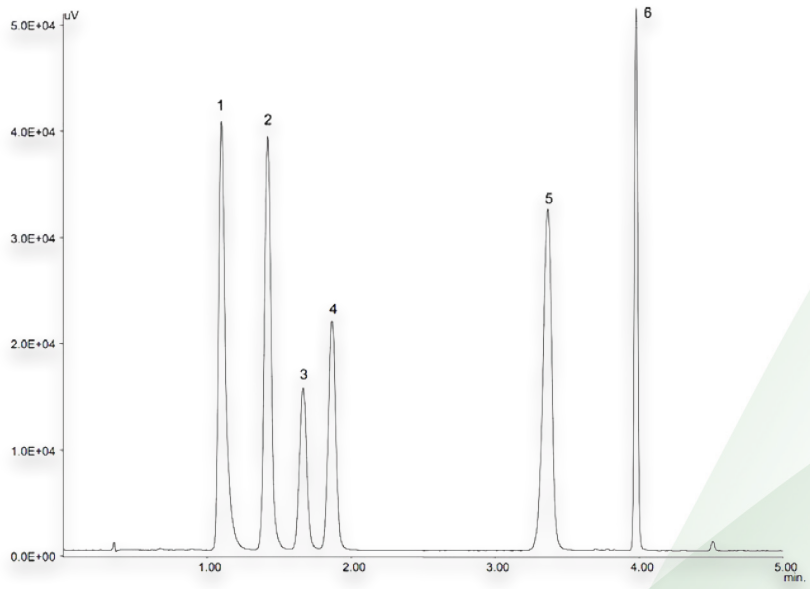

Figure 1. X-LC chromatogram of a standard mixture Peaks: 1 = adenosine triphosphate (ATP), 2 = adenosine diphosphaete (ADP), 3 = inosinic acid (IMP), 4 = hypoxanthine (Hypo), 5 = adenosine monophosphate (AMP), 6 = inosine (Ino) Chromatographicconditions: Column = X-Presspak AQ-C18-W (3.0 mm ID x 50 mmL,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

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

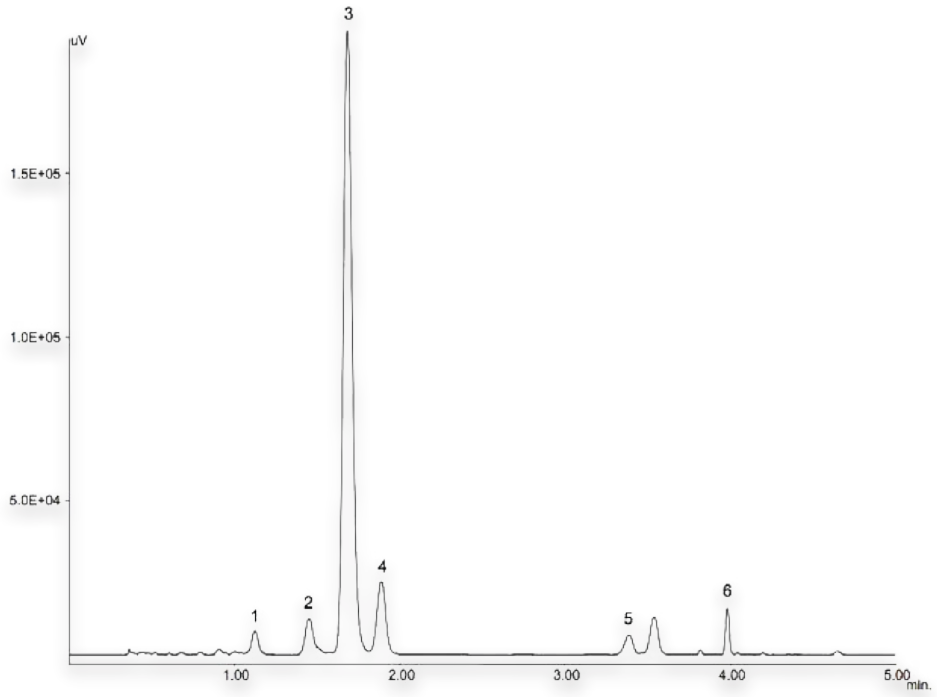

Figure 2. Figure 2. X-LC chromatogram of tuna sample. The pretreatment is describe in Experimental.The other conditions are the same as in Figure 1 caption.

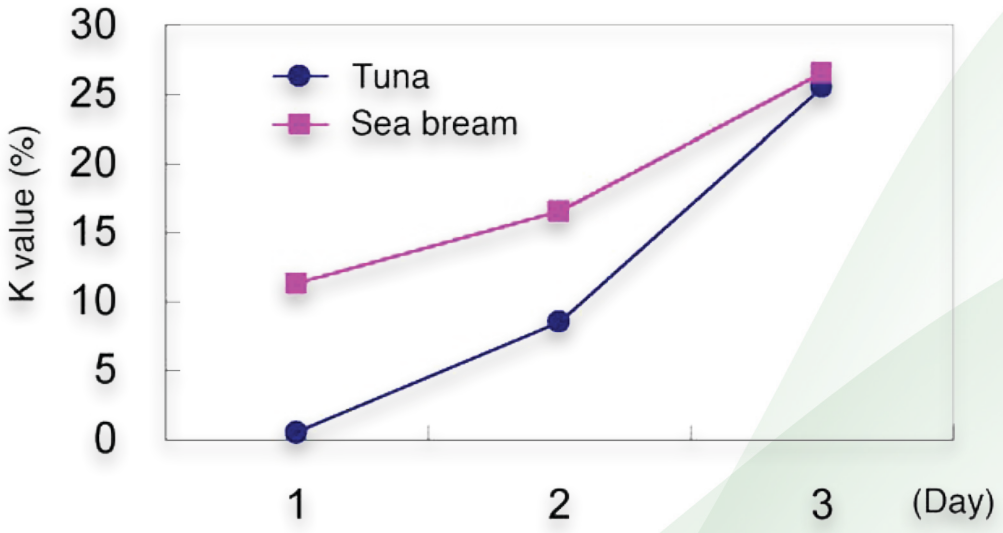

Figure 3. Relationship between preservation days and K values.

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