

Original article

Simultaneous determination of freshness and histamine of fish flesh

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Keywords: Fish; Freshness assessment; K-value; Freshness checker; Safety assessment; Histamine.

Received: 19 July 2017 / Accepted: 8 September 2017

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Introduction

Fish is an important nutritive component of the human diet as it provides protein, energy and many other functional components and thus helps sustain health. Concerning fish quality, fish deteriorate more quickly post-mortem than meat derived from terrestrial animals, so quick and simple methods are required to confirm fish freshness and quality. Fish freshness is influenced by various factors such as pre- and post-slaughter treatment (catching method, activity, handling, storage time) and preservation temperature [1]. Concerning fish safety, more than 50% of food intoxication caused by fish occurs because of histamine (Table 1) [2]. Histamine is well known as a substance causing allergy-like food poisoning after ingestion of scombroid fish that are not chilled adequately between harvest and consumption.

The authors propose that the freshness and histamine content of fish should be confirmed to ensure safe, tasty fish, and to produce safe processed food [2]. Here, we report a new method that can measure the freshness (K value) and histamine content of fish flesh with a single electrophoresis test paper.

Table 1. Number of foodborne disease outbreaks attributed to fish, United States, 1993–2008

Etiology	No. outbreaks	(%)
Bacteria	33	4.4
Biological toxin	227	30.3
Histamine	383	51.2
Parasites	1	0
Virus	8	1.1
Known etiology	669	89.4
Unknown etiology	74	9.9
Total	748	100

Materials and methods

Simultaneous determination of freshness and histamine were done as follows. First, the freshness of fish flesh is measured by the Freshness Checker (QS-SOLUTION), which is based on a paper electrophoresis method. After

measuring the freshness data of fish using the electrophoresis paper by UV detection, histamine is detected by spraying histamine-developing reagents on the paper.

Measurement of K value by the freshness checker

This is based on four steps: preparation of an extract from small pieces of fish (about 250 mg) with 600 μ l perchloric acid; application of 3 μ l extracts onto electrophoresis paper and separation of nucleotides and nucleosides + bases by electrophoresis at 800 V for 5 min; detection of spots by UV irradiation and digital imaging of two spots (spot A in the anodal position and spot B in the original position of sample loading; Fig. 1); and calculation of K value using a computer program (Fig. 1). Spot A includes nucleotides, such as ATP, ADP, AMP and IMP; and spot B includes nucleosides + bases, such as HxR and Hx. The computer program Spot Analyzer calculates the integration ratio of the two spots (spot size and density), according to eqn (1). In this way, K value can be calculated in less than 10 min.

$$K\% = \frac{[\text{Integration value of spot B}] \times 100}{[\text{Integration value of spot A+B}]} \quad \text{eqn (1)}$$

Detection of histamine on the same electrophoresis paper

After taking the freshness data of fish on the electrophoresis paper, the histamine detection was done by spraying of Pauly reagent. Histamine can be satisfactorily detected and completely separated from histidine, carnosine, and other Pauly-reagent-positive compounds.

Sample name : Fresh mackerel

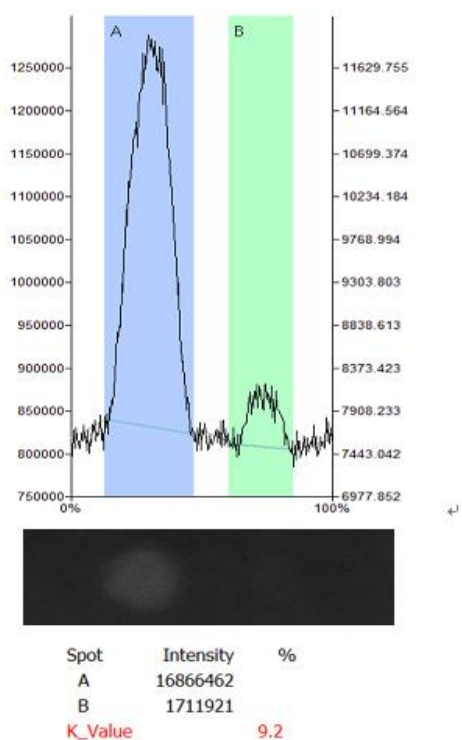


Fig. 1. K value estimation by Spot Analyzer.

References

1. Sato M, Gleadall IG (2014) Encyclopedia of Meat Science 2nd ed. Vol.2, Devine C, Dikemann M eds. Oxford: Elsevier; pp. 8–16
2. Sato M (2017) Reito 91: 356–361 (in Japanese)

Results and discussion

By this method, freshness (K value) and histamine can be measured in multiple fish and seafood samples simultaneously, at low running cost without tedious pretreatment, as shown in Figure 2. This means that seafood intoxication can easily be avoided and fish can be ingested and used for seafood processing safely.

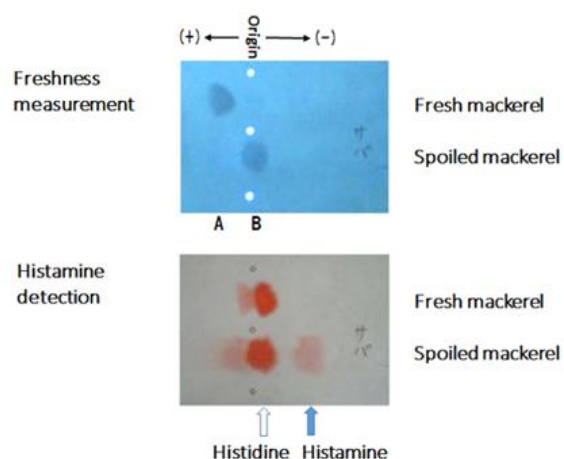


Fig. 2. Simultaneous screening of freshness and histamine on the same test paper.