

## **Validation of a simplified method for determination of K-value by interlaboratory study.**

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[Background] K-value is determined in many research institutes and testing laboratories as a fish freshness indicator. However, it was difficult to compare the K-value data from different laboratories because each laboratory used different determination procedure, reagents and apparatus. In addition, the conventional K-value measurement method was complicated and required skilled persons to perform accurate measurement. Therefore, in order to standardize the K-value determination method, simplification and validation were examined.

[Methods] To simplify the measurement method, the well-known conventional procedure was modified. An interlaboratory study to validate a simplified new method was designed based on the IUPAC protocol. As the test samples, frozen fish meat powder prepared from white muscle was packed in plastic bottles and distributed to each laboratory. The fish species selected considering white and red fish, natural and farmed fish, etc. According to a provided method, eleven laboratories quantified ATP-related compounds in the 10 test samples (5 pairs of blind duplicates) and calculated K-values.

[Results] The neutralization process of the perchloric acid extract was simplified by a two-step pH adjustment using alkaline solution and neutral phosphate buffer. In the interlaboratory study, specified statistical indices were calculated from K-value data reported from each interlaboratory. The discernible range of K-value ( $4s_R$ ) calculated from the reproducibility standard deviation ( $s_R$ ) was approximately 1% for high freshness samples (K-value 6-10%), approximately 2% for medium freshness samples (K-value 25-40%), and approximately 5% for low freshness samples (K-value 84%). These results were approved by a project committee composed of academic experts and others. Based on this method, a draft standard was submitted to the Ministry of Agriculture, Forestry and Fisheries (Japan), and "Test Method for Freshness (K-value) of Fish - High Performance Liquid Chromatography" was established as a Japanese Agricultural Standard in March 2022.

**Keywords:** K-value, standardization, interlaboratory study