# NEW FRESHNESS INDEX, METHOD AND DEVICE TO DETERMINE FRESHNESS STATUS OF MEAT AND FISH

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## I INTRODUCTION

There is a need for determination of freshness and monitoring of autolytic and microbiological changes in meat and fish by a simple, low-cost and reliable method and device. Our aim was to elaborate a method usable not only in well-equipped research laboratories but also "in the field", e.g., in industries, retail chains or even in households, using the principles of Fast Protein Liquid Chromatography (FPLC) on size-exclusion (SEC) columns. SEC separates molecules primarily by their particle size (molecular weight).

## II MATERIALS AND METHODS

***Samples*.** A variety of meat and fish samples (64) were studied: 1) fresh farmed fish (trout and carp); 2) the same fish, stored at -20° or thermally treated at +95 °C; 3) pork, beef, poultry; 4) minced pork; 5) minced pork with various ingredients of plant origin and 6) samples 4 and 5, cooked at ~200°C. Extracts were prepared with TRIS buffer (pH 8.2-8.4) in two parallels.

***Chromatographic methods***. FPLC experiments for estimation of delay time $\Delta t$ at SEC columns (Fig. 2) and LC-MS/MS on 1100 Series LC/MSD Trap-XCT of Agilent Technologies for identification of substances and quantification of inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx) were performed.

## III RESULTS AND DISCUSSION

FPLC chromatograms of meat or fish extracts on a size-exclusion gel column (SEC) consist of two main areas: a sharp peak at t = 0 that belongs to proteins and other high-molecular substances that is followed by a broad elution band (Fig. 1). The maximum of this broad band, mostly formed by merging of individual peaks of the nucleosides and their derivatives, such as ATP, ADP, AMP, IMP, Ino and Hx, shifts to longer retention times during the storage of a meat or fish sample mostly due to consecutive enzymatic decompositions ATP→ADP→AMP→IMP→Ino→Hx, accompanied by continuous reduction of the molecular weight of the substance (Fig. 1) [1].

The time interval between protein and nucleosides' peak maxima, called as the *Delay time* $\Delta t$, is the most important parameter determined by FPLC or, in this case by FPNLC (Fast Protein Nucleoside Chromatography) that is considered hereby as a new freshness or spoilage index [2].

There is a clear correlation between delay times $\Delta t$ and calculated standard freshness indices $K_i$ = ([Ino]+[Hx])/([IMP]+[Ino]+[Hx]) (Fig. 3). Analogical correlations were also established for freshness indices Fr, H, K, P and Q. All corresponding correlation formulas are embedded in the software of the device (Fig. 2) and can be used for fast calculation of freshness and taste indices.

Some other useful parameters of different foodstuffs can easily be derived from the measured delay time $\Delta t$. For instance, the ratio [IMP]/([Ino]+[Hx] can be considered as a parameter characterizing the taste of meat [3]. As it follows from the correlation in Fig. 3, at times $\Delta t \leq 150$ sec the ratio is $\geq 1$, i.e., respective meat or fish sample is relatively rich in IMP, a well-known flavour enhancer (E 630) and, hence, can be suitable for perception of meaty and brothy taste as well as for *haute cuisine*, e.g., umami dishes [3].

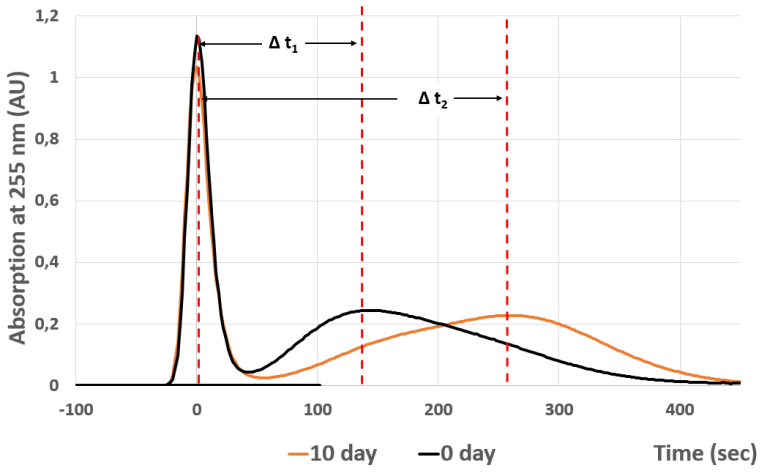
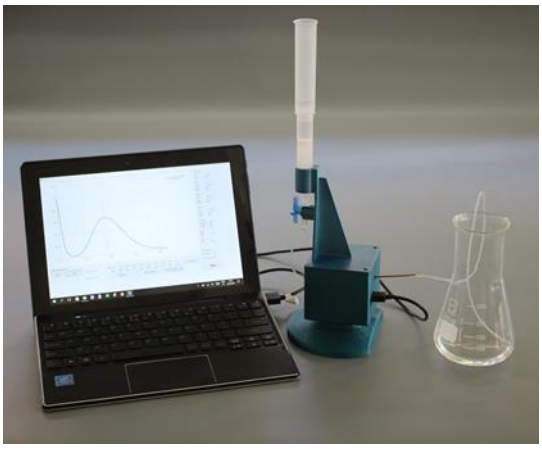



Fig. 1. FPNLC chromatograms (λ = 255 nm) of horse meat extracts within time interval of 10 days.



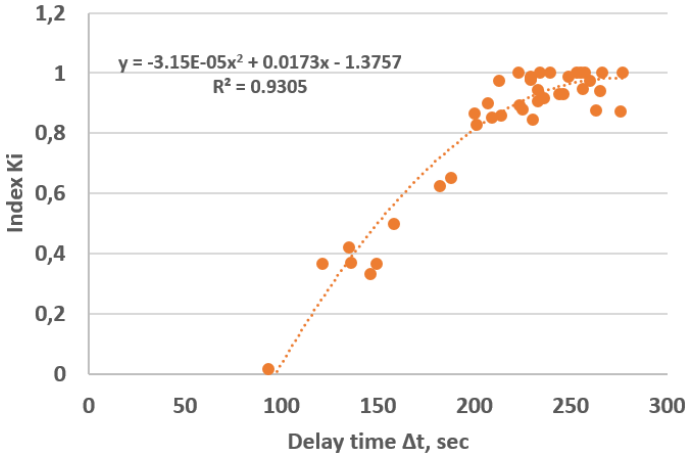



Fig. 2. Device for measuring of the delay times. Fig. 3. Universal equation and correlation in the coordinates "Delay time Δt – freshness index $K_i$", further used for the conversion Δt → $K_i$.

## IV CONCLUSION

New method and device can be used for various purposes connected with quality and safety of meat and fish.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