DOI: 10.58895/ksp/1000128686-3 erschienen in:
OCM 2021 - 5th International Conference on Optical Characterization of Materials, March 17th – 18th, 2021, Karlsruhe, Germany : Conference Proceedings
DOI: 10.58895/ksp/1000128686 | https://www.ksp.kit.edu/site/books/m/10.58895/ksp/1000128686/

A new approach for evaluation of meat freshness

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Abstract Adenosine triphosphate (ATP) and inosine monophosphate (IMP) dominate in fresh meat at the stage of autolysis, whereas ATP exists only during very first hours after slaughter. Hence, ATP is a highly suitable marker of absolute freshness of rapidly frozen meat/fish. Fast Protein Metabolites Liquid Chromatography (FPMLC) method was used to evaluate freshness of minced pork during storage and compared to results of standard methods -Volatile Fat Acids (VFA) and total count of bacteria. Good agreement between new FPMLC method and standard methods was achieved.

Keywords Meat freshness index, inosine monophosphate, hypoxanthine, fast liquid chromatography

1 Intoduction

Many meat and fish freshness/quality assessment indices are based on combination of concentrations of ATP breakdown products:

$$\mathrm{ATP} \rightarrow \mathrm{ADP} \rightarrow \mathrm{AMP} \rightarrow \mathrm{IMP} \rightarrow \mathrm{Ino} \rightarrow \mathrm{Hx}, \tag{1.1}$$

where ATP, ADP, AMP are adenosine tri-, di- and monophosphate respectively, IMP - inosine monophosphate or inosinic acid, Ino – inosine and Hx – hypoxanthine [1]. ATP and IMP dominate in fresh meat at

the stage of autolysis, whereas ATP exists only during very first hours after slaughter. Hence, ATP is a highly suitable marker of absolute freshness of rapidly frozen meat/fish. IMP is well-known endogenous taste enhancer (E630) and synergic component of umami taste [2]. Hx, on the contrary, lead to the bitter off-taste [3], which makes it a good sign of the onset of meat or fish spoilage. Increased concentration of Hx may also be a matter of health since hypoxanthine is a precursor of hyperuricemia and gout [4] or associated Lesh-Nyhan syndrome [5].

The first historical ATP-linked freshness index K was proposed by Saito et al. [6,7]:

$$K = \frac{[Ino] + [Hx]}{[ATP] + [ADP] + [AMP] + [IMP] + [Ino] + [Hx]} \tag{1.2}$$

Since ATP, ADP and AMP exist in remarkable concentration only during short period of first hours, Karube et al. proposed simplified index K_i for operation in the ordinary time scale of days [8]:

$$K_i = \frac{[Ino] + [Hx]}{[IMP] + [Ino] + [Hx]} \tag{1.3}$$

Both indices, K, and K_i, have been widely used in fish science [1,7], but considerably less for red meat [9], regardless of the fact that these ATP metabolites have been exploited for a long time as freshness markers [10–16]. One of the reasons can be that the indices change only in a limited range of values from 0 to 1 (or 0% - 100%) with no great or dramatic dynamics.

Furthermore, linking data between freshness indices and bacterial contamination of meat is deficient. Mostly researches on ATP metabolites and bacterial contamination have been done separately without any attempt to combining both into one more universal approach. Recently, a two-band freshness index (TBFI) was proposed to monitor bacterial contamination on chicken meat surface using hyperspectral imagery technique [17]. It is crucial to look for correlation of freshness indices and bacterial contamination to expand methodological and technical capabilities in establishment of freshness and early spoilage indications of foods.

The aim of this work was to study correlations of K_i indices and standardized meat evaluation methods (e.g., Volatile fatty acids VFA and bacterial contamination), validating the FPMLC method in process.

2 Materials and methods

Sampling and materials The study was performed in two sequential experiments. Pork sirloin, one-day-after-slaughter, was minced and samples were packed according to sampling plan in two different conditions: air and vacuum and were stored at 2 – 4 °C for 14 days. Samples were analyzed every other day according to the schedules, 8 times in total.

Volatile fatty acids (VFA), total count of aerobic microorganisms and Pseudomonas spp. were determined by the Estonian Veterinary and Food Laboratory (EVFL). The FPMLC device designed by Ldiamon AS has been used for determination of ATP metabolites [18,19]. Determination of ATP metabolites concentration was carried out by LC-DAD (MS) and determination of lipid oxidation level by the TBARS method in Estonian University of life Sciences.

pH determination Measured in homogenized mixtures of 1 g of sample and 9 mL of distilled water with Consort C833 digital pH-meter (Consort, Turnhout, Belgium) at room temperature. Calibration of pH meter was regularly checked.

Determination of ATP metabolites by FPMLC Mixture of 2 g of minced pork with 6 ml TRIS buffer (pH 8.0) in 15 ml test tube was shaken for 11 minutes in a rotator Biosan Multi RS60 (BioSan, Riga, Latvia) and filterd with syringe filter (Whatman, 0.45 µm.). 6 drops of extract was dropped into the PD-10 column of the FPMLC device.

Determination of ATP metabolites concentration by LC-DAD (MS) The liquid chromatographic analysis of meat extracts was carried out by a 1290 Infinity system (Agilent Technologies, Waldbronn, Germany) coupled to an Agilent 6450 Q-TOF mass spectrometer equipped with a Jetstream ESI source. Samples were subjected to a Zorbax 300SB-C18 column 2.1×150 mm; 5 µm, (Agilent Technologies) kept at 40 °C. For the separation of compounds, a gradient of 0.1% of formic acid in water (A) and 5% of water in acetonitrile (B) was used as follows: 0.0 min 1% B, 3.0 min 1% B, 3.01 min 99% B, 11.1 min 99% B, 11.01 min 1% B, regeneration time 8 min. The eluent flow rate was set to 0.3 mL/min

and the injection volume size was 2.5 µL. Mass-spectrometer was working in the negative ionization mode in the mass-to-charge ratio (m/z) range of 100–3200 Da. UV absorbance was measured at 250 nm. Data acquisition and initial data processing were carried out by MassHunter software (Agilent Technologies).

The identification of IMP, Ino and Hx in samples was confirmed by comparing MS/MS and UV spectra with those of analytical standards. IMP, Ino and Hx were quantified by UV absorption at 250 nm using external calibration curve method. Methanolic standard solutions with concentrations of 3.125, 6.25, 12.5, 25, 50 and 100 µM were prepared for all three analytical standards. Calibration curves were characterized by a high correlation coefficient ($R^2 = 1$).

Validation The chain of parallel measurements and comparison of VFA and FPMLC results were considered as validation procedure of FPMLC method. For this purpose, a "clone" samples were tested at the EVFL with the standard method VFA [20]. Official protocols from the EVFL have been compared with results of synchronized measurements carried out by University of Life Sciences and Ldiamon AS.

3 Results and discussion

FPMLC chromatograms of meat consist of two main parts: the sharp protein peak and broad post-protein band (Figure 3.1) that is formed by merging of individual peaks of the main nucleotide actors. During the storage of meat, the ATP is decomposed by the enzymes to the metabolites with smaller molecular weight, as a result, the retention time of the metabolites increases. The major operand of FPMLC analysis - Time, measured in seconds, is the time interval between retention time of the flat band of metabolites and sharp protein peak in the FPMLC chromatograms (Figure 3.1).

The changes in concentrations of ATP degradation products - IMP, Ino and Hx, that occurred in minced pork during air and vacuum storage for 14 days at 2 – 4 °C with correspondently calculated K_i indices are shown in Figure 3.2. In air storage decreasing in concentration of IMP during the meat storage took place more rapidly than in vacuum,

degression 3,14 and 1,42 times, respectively. Changes in K_i indices were steeper in air stored samples for first 7 days of meat storage.

Correlation of new FPMLC method and LC-DAD/MS was satisfying (Figure 3.3). Correlations for both FPMLC indices – Time and K-value with K_i values determined by LC-DAD/MS were linear, with values of R^2 respectively 0,83 and 0,86.

Compatibility of FPMC method and bacterial contamination was good for both storage conditions air and vacuum (Figure 3.4). Correlations of indices Time and K-value with total count of bacteria *Pseudomonas spp.* was linear with values of R^2 0.93-0.94 and 0.79-0.81 respectively for air and vacuum stored samples.

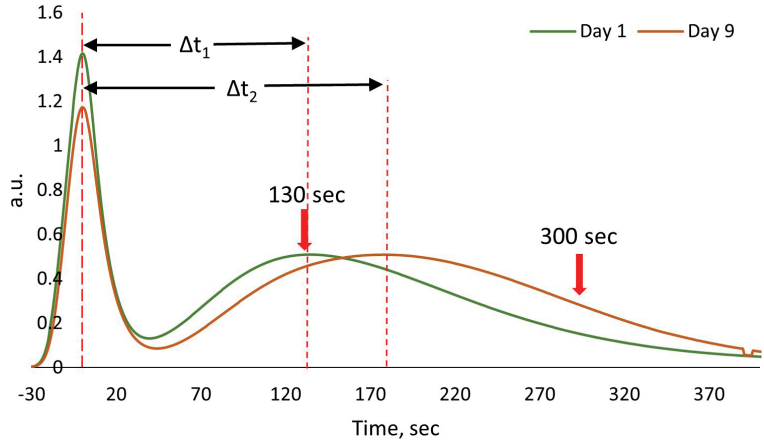

Figure 3.1: Comparison of two FPMLC chromatograms produced at days 2 and 9 during storage of a pork meat.

Validation Validation process of FPMLC method took place in strictly parallel mode of progression of the indices VFA and Time in cooperation with EVFL (Figure 3.5).

The simultaneous steep rises after the 9th (pH=5.6) or 11th (pH=5.3) days mark the end of changes in condition of meat caused by autolysis and onset of changes caused by bacterial activity. For more acidic pork (Figure 3.5b) the level of VFA in the flat interval of predominantly autolytic transformations is somewhat higher than for the meat of pH 5.6.

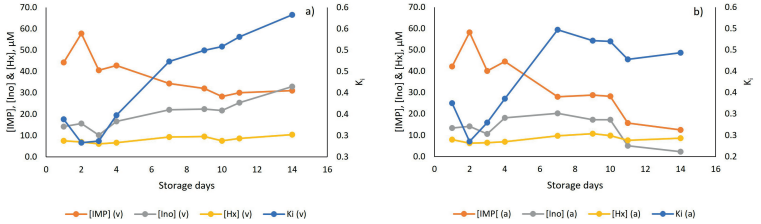

Figure 3.2: Changes in concentrations of ATP metabolites – IMP, Ino and Hx, and corresponding K_i values during the storage of minced pork in vacuum (a) and air (b) at 2 – 4 °C.

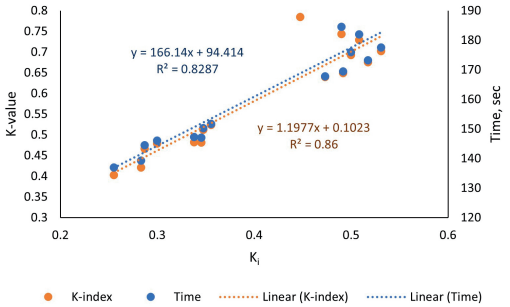

Figure 3.3: Correlations between K_i values determined by LC-DAD (MS) and main indices of FPMLC – Time and K-value for minced pork stored in vacuum and air at 2 – 4 °C.

Therefore, the maximal level of VFA indicated for the pork pH 5.6 on the day 14 is remarkably higher than for acidic one, i.e., 19.2 and 11.78 mg KOH/100g, respectively. In both cases, at the onset of the steep rise the meat had already specific heavy odor and slime.

Correlation coefficients for the paired curves are high: r = 0.955 (pH=5.6) and r = 0.93 (pH=5.3) sustaining credibility and reliability of the new FPMLC method.

4 Conclusions

Results of FPMLC method were in a good agreement with standardized methods of VFA and total bacterial count. Both indices of FPMLC

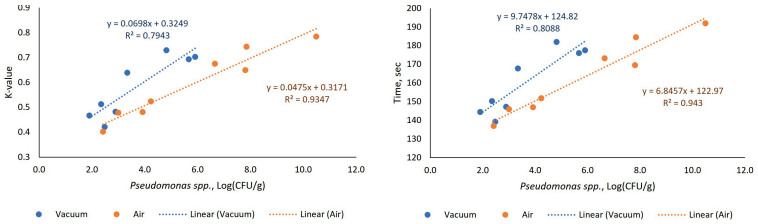

Figure 3.4: Correlations of FPMLC operands – K-value and Time with total count of bacteria *Pseudomonas spp.* (Log (CFU/g)) for minced pork stored in vacuum and air at 2 – 4 °C.

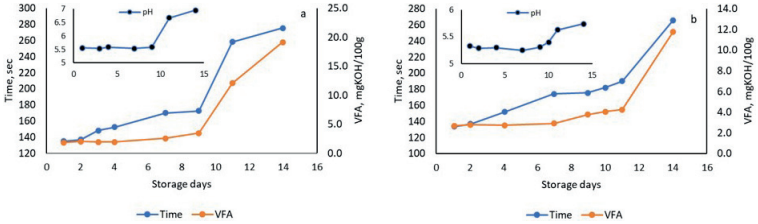

Figure 3.5: Changes in VFA and FPMLC indices Time for air stored minced pork at 2 – 4 °C with different initial pH levels - pH=5.6 (a) and pH=5.3 (b).

– Time and K-value were in a good correlation with bacterial growth of *Pseudomonas spp.*.

FPMLC method has promising validity in relation to minced pork freshness control and needs to be studied further with other subjects of meat or fish. The FPNLC method can be applicable for the selection of the raw meat or fish of the highest quality or cold chain management for special control of frozen meat and fish in suspicious cases.

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