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〈研究ノート〉

The trial for evaluation of chicken freshness using near infrared spectroscopy

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Summary

The freshness of chicken breast muscle was non-destructively determined using near-infrared (NIR) spectroscopy after comparison of the degradation characteristics for chicken breast muscle and pork Boston butt under storage at 4°C in consideration of changes in K values and nucleic-related compound content. The NIR diffuse reflectance spectra of chicken samples were measured using a spectrophotometer with a fiber optic probe, and multiple linear regression was carried out based on second derivative spectra and K values.

The rate of chicken meat degradation was quite rapid, with decomposition from inosine (HxR) to hypoxanthine (Hx) being observed within seven days of the start of storage. HxR increased steadily in pork, for which a viable calibration equation has been established as previously reported. The best multiple correlation coefficient of 0.83 was obtained from multiple linear regression analysis using K values of less than 50% as an objective variable (composed of wavelengths of 1,156, 835 and 1097 nm). As the ratio of HxR and Hx in K values changed greatly when K values were over 50%, it can be inferred that determination of K values using NIR spectroscopy might be more applicable to chicken breast muscle with K values significantly below 50%.

Keywords：near infrared spectroscopy, chicken, freshness, K value, nucleic acid related compounds

Introduction

Chicken muscle loses its freshness faster than beef or pork muscle, and its quality is thereby significantly affected.[1)] To evaluate the quality of chicken, changes in pH, volatile basic nitrogen and live bacterial count are measured as indicators of preservative condition. However, these indices are inadequate for determining the extent of deterioration before the onset of putrefaction. Against such a background, the suitability of K values[2)], which are used to indicate fish freshness, for determining the freshness of chicken has been evaluated.[1), 3-6)] The results showed that freshness is an important indicator in determining the extent of autolysis, or aging, in chicken. A quick method of chicken freshness evaluation involving a non-destructive approach is needed to enable quality determination for commercial distribution.

In the area of food freshness evaluation, three papers[7-9)] have reported on the non-destructive determination of K values to indicate the freshness of fish[7, 8)] and pork[9)] using near-infrared (NIR) spectroscopy. One of these studies involved bonito and tuna[7)], and the others focused on mackerel[8)] and pork.[9)] The results indicated the potential for practical evaluation of freshness using K values.

In this study, NIR spectroscopy was applied to measure the K values of chicken in real-time determination of freshness.

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Materials and Methods

Production of chicken samples

Breast muscles obtained from chickens slaughtered in the morning at Shizuoka Broiler Center were cut into pieces measuring 3 × 3 × 1 cm. The numbers of pieces cut from breast muscles were 40, 43 and 50 (Nos. 1 – 3). They were put into polyethylene bags with a thickness of 0.04 mm and stored in a refrigerator at 4°C for individual grading over seven consecutive days (Days 0 – 7), thereby creating three stored samples with wide K value distributions. The samples were then used for NIR analysis and subsequent quantitative analysis of ATP-related compounds for determination of K values using high-performance liquid chromatography (HPLC) as outlined below.

Chemicals

Perchloric acid (PCA), phosphoric acid, potassium hydroxide, potassium carbonate anhydrous, sodium dihydrogenorthophosphate, adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

NIR evaluation of chicken breast muscle

NIR diffuse reflectance spectra were evaluated using a Model 6250 spectrophotometer (NIRECO-NIR Systems, Tokyo, Japan) with a coaxial fiber optic probe (680 – 1,235 nm) fixed and settled vertically toward chicken samples set on the upturned bottom of a piece of white ceramic chinaware.[9] The distance between the contact point (lower end) of the probe and the surface of the chinaware was 1 cm. The whole measurement section was covered with a light-shielding cloth during NIR evaluation with 50 scans conducted at 20°C with the fiber optic probe set on 3 – 4 points of the large planes for each chicken sample after a ceramic plate with a thickness of 6.5 mm was evaluated for reference.

Determination of ATP-related compound content using HPLC

After NIR evaluation, ATP-related compound content in each piece of meat was measured as described here. First, two 1.5-gram portions of each sample were homogenized using a Model BM-2 (Nihonseiki Kaisha Ltd., Tokyo, Japan) in test tubes with 4.0 ml of iced 5.0% PCA for 90 seconds each, followed by centrifugation at 3,000 rpm for 5 minutes. Second, 1.0 mL of supernatant from each sample was mixed in a test tube, and the mixture was neutralized to pH 6.8 with 40 μL of 10 M potassium hydroxide and 160 μL of 1.0 M potassium carbonate, followed by centrifugation at 3,000 rpm for 3 minutes. Third, 0.5 mL of the supernatant was mixed with 2.0 mL of distilled water and then passed through a 0.45 μm membrane filter (DISC-13, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) before injection. Finally, a 10 μL portion of test solution was injected into an Asahipak GS320HQ (7.6 × 300 mm, Shodex, Tokyo, Japan) column eluted with 0.2 M of sodium dihydrogenorthophosphate adjusted with 0.2 M phosphoric acid to pH 3.0. The flow rate of the eluate was 0.5 ml/min and the column was at room temperature. The eluate was monitored with UV absorption at 250 nm, and the ATP-related compounds were analyzed by comparing the retention times of HPLC peaks between samples and authentic compounds. The freshness of the muscle was judged from the K value as defined by Equation[2] :

$$K\ value\ (\%) = (HxR + Hx) / (ATP + ADP + AMP + IMP + HxR + Hx) \times 100$$

Statistical analysis

NIR spectral analysis was conducted using Spectra analysis software version 3.27 (NIRECO-NIR Systems) with the measurement device, and multiple linear regression was carried out based on second derivative spectra and the K values as determined from chemical analysis. The data from the three sets of chicken samples (Nos. 1 – 3) mentioned above were used in the statistical analysis for calibration.

Results and Discussion

K values and IMP, HxR and Hx content in stored chicken samples compared with those of pork

The relationships between K values and percentages of IMP, HxR and Hx among all ATP-related compounds in stored pork[9] and chicken samples are shown in Figs. 1 (1A – 1C) and Figs. 1 (2A – 2C), respectively. K values and percentages of IMP, HxR and Hx among all ATP-related compounds in stored pork[9] and chicken are shown in Figs. 2 (1A – 1D) and Figs. 2 (2A – 2D),

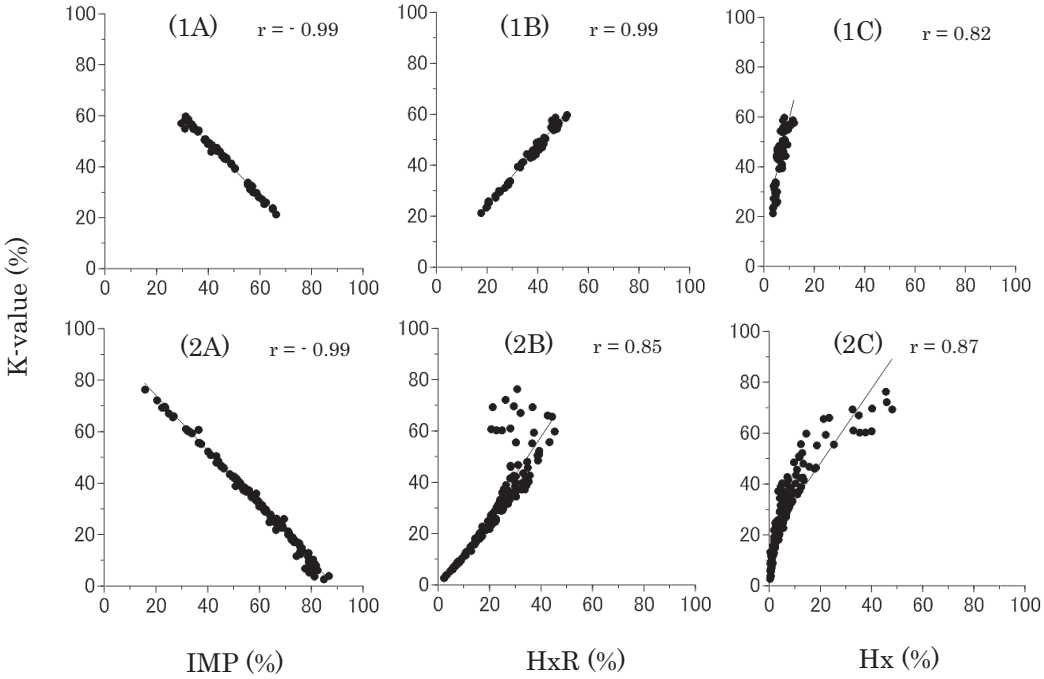

Fig. 1 Relationship between K values and percentages of IMP, HxR and Hx among all ATP-related compounds in stored pork and chicken samples. Figures (1A – 1C) and (2A – 2C) show data for pork and chicken samples, respectively. Each dot represents the mean value of two samples from the same piece of meat. 1A – 1C, n=67; 2A – 2C, n=133.

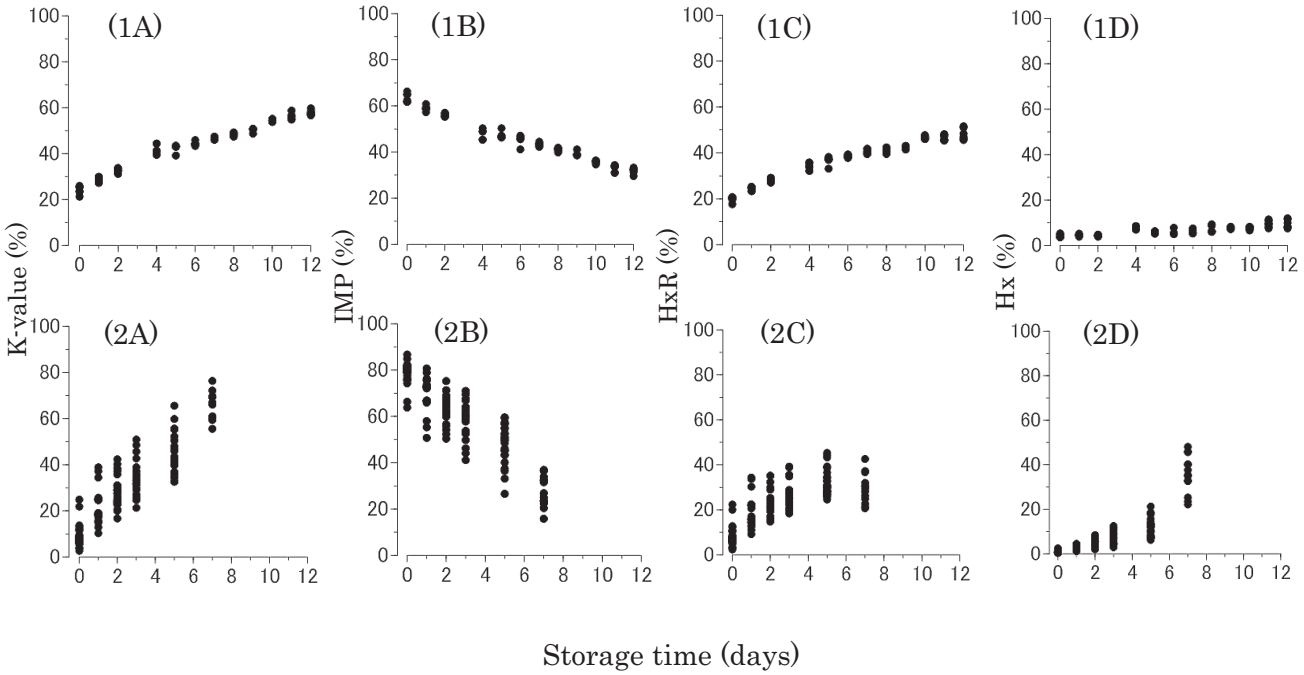

Fig. 2 K values and percentages of IMP, HxR and Hx among all ATP-related compounds in stored pork and chicken samples. Figures (1A – 1D) and (2A – 2D) show data for pork and chicken, respectively. Each dot represents the mean value of two samples from the same piece of meat. 1A – 1D, n=67 ; 2A – 2D, n=133.

respectively. Figs. 1 (1A – 1C) and Figs. 2 (1A – 1D) are based on previously reported data.[9)]

In Fig. 1 (1B), a high correlation between K values and HxR as well as IMP (Fig. 1 (1A)) is observed, while a lesser correlation is seen between K values and HxR in the chicken samples containing high K values of more than 50% (Fig. 1 (2B)). On the other hand, a high correlation (0.965) between K values and HxR was also reported in bonito.[7)] Figure 2 (2C) shows that HxR values began to decrease seven days after the start of preservation, while Hx, which is a product of HxR degradation, suddenly began to increase (Fig. 2 (2D)). The rate of change in K values in chicken was quite rapid compared with that in pork, and decomposition from HxR to Hx started within seven days whereas the amount of HxR in pork continued to increase constantly during storage (Fig. 2 (1C)). In pork and bonito[7)] , those were presumed to be applicable for NIR analysis, HxR amounts increased with storage time. However, the amount of HxR in chicken did not increase in proportion with storage time (Fig. 2 (2C)). These results suggest that if NIR spectra are influenced by HxR, the correlation coefficient of the calibration equation might be low, especially with samples whose K values exceed 50%.

Formulation of a calibration equation using multiple linear regression

The calibration equation created from multiple linear regression analysis was based on second derivative spectra and K values determined by chemical analysis, and the results are shown in Table 1. For the three wavelengths selected, multiple correlation coefficients of about 0.8 were obtained using K values of less than 50% for calibration. Removing samples in which the K value exceeded 50% increased the multiple correlation coefficient of each sample set as observed in data nos. 1 – 1a and 2 – 2a.

These results suggest the applicability of the calibration equation for determining K values to chicken breast muscle with low K values not exceeding 50%.

Finally, in terms of the property of chicken meat, it can be difficult to maintain the freshness of it with K values of less than 50% because these values stop increasing only at temperatures less than -30°C.[1)] In a practical context, K values exceeding 50% were found in several samples of commercially available fresh chicken and imported frozen chicken.[1, 4)] Essentially, it is quite possible for chicken meat to have a significant range of K values and extents of putrefaction. For example, chicken meat with fairly high K values but no extent of putrefaction can be produced. In addition

Table 1.　Results of calibration for determining K values (%)

Sample Set		K value (%)*2			Wavelength selected (nm)			R*4	SEC*5 (%)
		Range	Average	SD*3	λ_1	λ_2	λ_3		
No. 1	(n = 40)	6.8 – 76.3	35.6	18.5	838	1,154	723	0.6747	13.5
No. 1a*1	(n = 31)	6.8 – 48.0	27.7	11.9	1,156	835	1,097	0.8343	6.50
No. 2	(n = 43)	3.8 – 72.2	37.1	19.4	962	1,092	—*6	0.5605	15.6
No. 2a*1	(n = 32)	3.8 – 48.5	28.4	14.2	960	1,204	—*6	0.6075	11.2
No. 3	(n = 50)	2.7 – 43.5	22.8	10.5	870	959	720	0.7406	7.05

*1 No. 1a, 2a: sets from which samples with K values exceeding 50% were eliminated.
*2 K value (%): chemically determined values of sample sets.
*3 SD: standard deviation.
*4 R: multiple correlation coefficient.
*5 SEC: standard error of calibration.
* — : wavelengths not selected due to mechanical error.

to examining methods of determining chicken freshness, it is also quite important to determine the relationship between K values and the chicken quality demanded by consumers.

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〈研究ノート〉

近赤外分光法の利用による鶏肉鮮度評価の試み

The trial for evaluation of chicken freshness using near infrared spectroscopy

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要　旨

食肉の熟成に要する時間は、家畜の種類によって異なり、牛肉や豚肉には数日を要するが、鶏肉は死後変化が非常に速く数時間で熟成することから、いわゆる朝引きの肉が美味とされている。その速やかな死後変化の度合いをとらえるには、揮発性塩基態窒素量や生菌数の増大などで検知される初期腐敗、それよりも前の段階の変化を追う必要があり、これまで魚類の鮮度指標であるK値の鶏肉への応用が検討され、その有効性が示されてきた。鮮度判定恒数K値を非破壊的に迅速に測定できると、死後変化が速やかに起こる鶏肉の品質管理に、より役立つものと考えられる。

本研究では、朝引きの鶏胸肉を入手し、４℃貯蔵により、K値にして３–76%の肉片を合計133片作成して３つの試料群（No.1–３）とし、それらを光ファイバープローブ（測定範囲：680-1,235 nm）を搭載した近赤外分光計により測定するとともに、湿式分析によりATP関連物質の含有量とK値を求め、先に近赤外分光法によるK値測定の可能性を報告した豚肉のデータと比較して、鶏肉の変化の特徴を探った。鶏肉においては、きわめて速やかなK値の上昇が認められるとともに、K値50%以上の試料にはイノシン量とK値の間に相関が認められず、豚肉に確認されカツオにも報告されているK値とイノシン間の極めて高い相関は認められなかった。すなわちイノシンには、経時的な増加と、引き続いての減少の傾向が見られ、イノシンの分解産物であるヒポキサンチンには、イノシン減少時に、より増加する傾向が確認された。このことから、熟成に数日を要する豚肉と、数時間しか要さない鶏肉では、イノシンの安定性にも違いがあり、鶏肉においては、急激な死後変化の中で、イノシンのヒポキサンチンへの分解も速やかに進むこと、そしてこの特性が近赤外分光分析の精度にも影響する可能性が推察された。

次に、装置付属の解析ソフトを用いて二次微分スペクトルとK値データを重回帰分析することにより、鶏肉の鮮度推定式の作成を試みた。上述の３つの試料群（No.1–３）のすべての肉片を用いての解析では、全試料のK値が50%以下の試料群No.3において、二次微分スペクトルの波長820, 959および720 nm選択時に、較正方程式の重相関係数は最高値の0.74であった。選択される波長がイノシンの影響を受けているのであれば、先に述べたように、K値50%以上の試料では、K値とイノシン量間に相関がないために、誤差は大きくなると考えられる。そのため、試料群No.1とNo.2からK値50%以下のデータを抽出し、再度、重回帰分析を試みた。結果として、双方ともに重相関係数は上昇し、試料群No.1（重相関係数0.67）からの抽出群において、二次微分スペクトルの波長1156, 835および1097 nmの選択時に、較正方程式の重相関係数は0.83という高い値が得られた。

以上のことから、鶏肉鮮度の近赤外分析は、イノシンとヒポキサンチンのバラツキの影響を受けにくいK値50%以下の肉において、実用の可能性が高いと考えられた。

一方、既に報告されているように、鶏肉のK値の上昇を止めるには－30℃以下での保蔵が必要となるため、それ以上の温度での保蔵では、様々な劣化度の鶏肉が生じることになる。すなわち細菌による腐敗は受けていないものの、K値が非常に高い鶏肉も生じることになり、市販鶏肉や輸入冷凍鶏肉には、K値50%以上のものがあることも報告されている。鶏肉において、上昇速度もバラツキも大きいK値であるが、鶏肉に求められる品質との関係について、多角的な知見が併せて求められる。

キーワード：近赤外分光法，鶏肉，鮮度，K値，核酸関連物質