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〈研究ノート〉

Non-destructive determination of K values in pork using near infrared spectroscopy

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Summary

Non-destructive determination of freshness of pork Boston butt was performed using near infrared (NIR) spectroscopy. NIR diffuse reflectance spectra of pork samples were measured using a spectrophotometer with a fiber optic probe, and multiple linear regressions were carried out based on second derivative spectra and K values, percentages of IMP and inosine.

The best calibration equation for estimating freshness was obtained through a multiple linear regression analysis using K values as an objective variable, composed of two wavelengths of 713 and 1,181nm. Following validation using other sample sets, it was found that the equation might be more applicable to pork Boston butt that has a low K value of less than 40%.

Keywords：near infrared spectroscopy, non-destructive, pork, freshness, K value, nucleic acid related compounds

Introduction

It's well known that meat quality is improved by so-called post-rigor conditioning, which leads to increases in tenderness, taste and flavor intensity.[1)] As a result, freshness is an important indicator in determining the extent of autolysis, or aging, in pork. A quick method of evaluation of pork freshness using a nondestructive approach is required to determine quality for commercial distribution.

In terms of evaluating the freshness of meat, two papers[2, 3)] have reported on the nondestructive determination of K values[4)] to indicate fish freshness using near-infrared (NIR) spectroscopy. One of the studies involved bonito and tuna[2)], and the other focused on mackerel.[3)] Both indicate the potential for practical estimation of freshness.

Meanwhile, Takahashi[5)] and Horiuchi *et al.*[6)] both reported that K values may be suitable for evaluating pork freshness because of correlations between storage times and the K values of stored meats.

In this study, we applied NIR spectroscopy to find K values in order to indicate the freshness of

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pork with the aim of enabling its practical use in the real-time determination of meat quality.

Materials and Methods

Production of pork samples

Frozen pork Boston butt from three animals, one from a Duroc pig and the others from crossbred (WLD) pigs, which were frozen 24 hours after slaughter (Shizuoka Prefectural Research Institute of Animal Industry Swine & Poultry Research Center) and packed in plastic film, were thawed in running water and then cut into pieces (3×3×1cm). The number of pieces cut from each butt was 76 (Duroc pig: Sample Set No. 1), 41 (WLD pig: Sample Set No. 2) and 46 (WLD pig: Sample Set No. 3). The pieces of meat were put in polyethylene bags with a thickness of 0.04 mm, deaerated and stored in a refrigerator at 4°C for individual grading over 12 consecutive days (Days 0–12) in order to prepare three sets of stored samples with wide K value distributions. They were then used as samples for NIR measurement 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 measurement of pork Boston butt

NIR diffuse reflectance spectra were measured 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 a pork sample set on the upturned bottom of a piece of white ceramic chinaware (Fig. 1). The distance between the contact point (lower end) of the probe and the surface of the chinaware was 1 cm. The measured section was covered with a light-shielding cloth during NIR measurement with a scanning frequency of 50 times, which was conducted at 20°C with the fiber optic probe set on both of its large planes for each pork sample after a ceramic plate with a thickness of 6.5 mm was measured for reference.

Determination of ATP-related compounds using HPLC

After NIR measurement, ATP-related compounds in each piece of meat were measured as described here. First, a couple of 1.5-gram portions of each sample were homogenized using a Model BM-2 unit (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 10 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 70 μL of 10 M potassium hydroxide and 320 μL of 1.0 M potassium carbonate, followed by centrifugation at 3,000 rpm for 5 minutes. Third, 0.5 mL of the supernatant was mixed with 2.0 mL of distilled water and then filtered through a 0.45 μm membrane filter (Nacalai

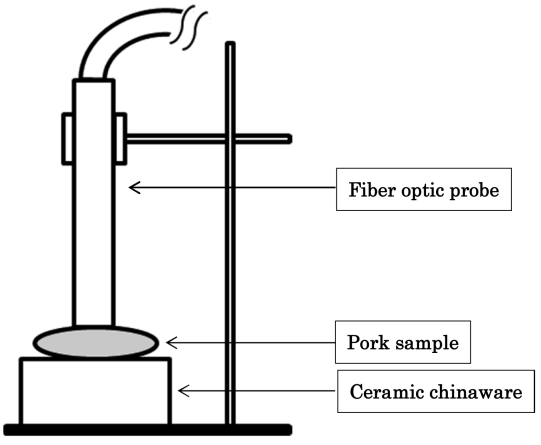

Fig.1　Measurement section of a model 6250 spectrophotometer.
The whole of this section was covered with light-shielding cloth during NIR measurement.

Tesque, Kyoto, Japan) before injection. Finally, a 10 μL portion of the 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 the equation[4)]

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

Statistical analysis

NIR spectra 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, or percentages of IMP and HxR, determined from chemical analysis. The data from the three sets of pork samples (Nos. 1–3) mentioned above were used in turn in the statistical analysis for calibration and validation.

Results and Discussion

Stored pork sample K values and IMP, HxR and Hx content

The data from the chemical analysis of the sample sets are shown in Table 1. The percentages of IMP, HxR and Hx represent values out of all ATP-related compounds because hardly any of other compounds such as ATP, ADP and AMP was detected in the pork samples.

The distribution of K values in pork samples stored for 0 to 12 days at 4° C are shown in Figure 2 (1A – 3A). These were 21.3 – 59.7% as shown in Figure 2 (1A), 29.6 – 54.7% as shown in Figure 2 (2A), and 23.2 – 58.2% as shown in Figure 2 (3A). The values increased with storage time, and a similar tendency was observed in all three sample groups (Nos. 1 – 3), which included pork from two different pig species.

Figure 2 (1B – 3B) shows temporal changes in K values and percentages of IMP, HxR and Hx content in the same samples shown in Figure 2 (1A – 1C). The values, which were averages of 4 – 6 samples, were 31.8 – 63.7% for IMP, 21.5 – 48.3% for HxR, and 4.3 – 9.6% for Hx as shown in Figure 2 (1A); 36.2 – 55.3% for IMP, 28.6 – 47.3% for HxR, and 4.5 – 6.4% for Hx as shown in Figure 2 (2A); and 33.1 – 59.4% for IMP, 23.7 – 48.7% for HxR, and 3.9 – 8.2% for Hx as shown in Figure 2 (3A), with the time course of the average K value. In this series of graphs, tendencies similar to those

Table 1. Analytical data from the sample sets used to determine K, IMP, HxR and Hx values (%) in pork Boston butt using near-infrared spectroscopy.

Sample set		K value (%)	IMP (%)*1	HxR (%)*1	Hx (%)*1
No. 1 (n = 76)	Range	21.3 – 59.7	31.8 – 63.7	21.5 – 48.3	4.3 – 9.6
	Average	43.5	45.1	36.9	6.6
	SD*2	10.6	10.1	9.0	2.0
No. 2 (n = 41)	Range	29.6 – 54.7	36.2 – 55.3	28.6 – 47.3	4.5 – 6.4
	Average	44.8	44.3	39.0	5.8
	SD	7.4	6.9	6.6	1.0
No. 3 (n = 46)	Range	23.2 – 58.2	33.1 – 59.4	23.7 – 48.7	3.9 – 8.2
	Average	41.4	47.2	35.4	6.0
	SD	9.8	8.8	8.3	1.7

*1 IMP(%), HxR(%), Hx(%): percentages of IMP, HxR and Hx in whole ATP-related compounds.

*2 SD: standard deviation.

reported in previous studies[1, 5, 6] can be observed in the changes in IMP and HxR. In the samples at 0 days (considered to be in almost the same condition as meat 24 hours after slaughter in terms of the extent of autolysis), the IMP values were the highest for the whole storage period, while hardly any ATP, ADP or AMP (precursors of IMP) was detected in the samples at that time. The values subsequently decreased gradually with storage time. In contrast, HxR values were more than 20% of all ATP-related compounds at 0 days (i.e., 24 hours after slaughter). The values subsequently increased throughout the storage period in a pattern similar to that of the K values. This result suggests that HxR values significantly influenced K values at least for a storage duration of up to 12 days in same-temperature conditions. However, for storage periods longer than those chosen for this study, subsequent decreases in HxR and increases in Hx have also been observed.[1, 6, 7] As a result, it can be suggested that K values are more appropriate than HxR percentages for judging the freshness of pork (i.e., the extent of autolysis) because K values depend on the total value of Hx (%) added to HxR (%).

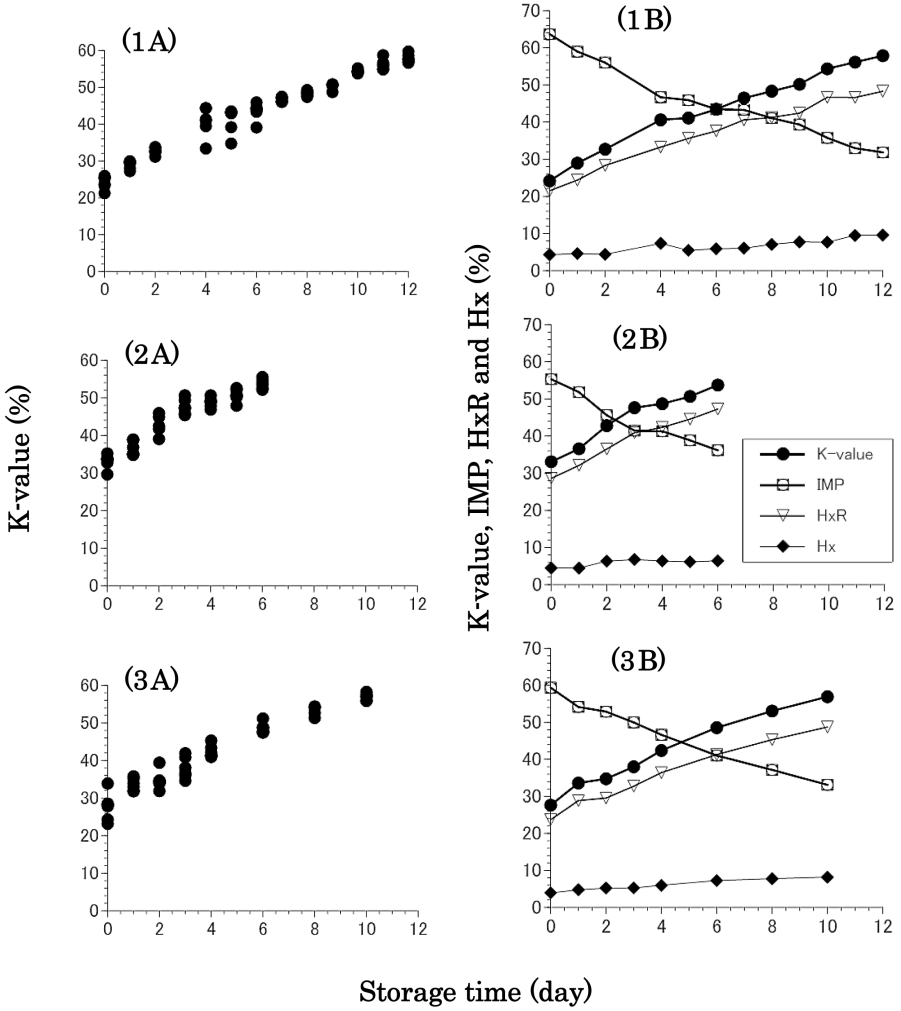

Fig.2　K values and percentages of IMP, HxR and Hx among all ATP-related compounds in stored pork samples. Figures (1A) and (1B), (2A) and (2B), and (3A) and (3B) show data for Sample Set No. 1 (Duroc pig), No. 2 and No. 3 (WLD pig), respectively.
Each symbol in Figures (1A), (2A) and (3A) represents the value of one pork sample, while each symbol in Figures (1B), (2B) and (3B) represents the mean value of four to six samples.

Preparation and evaluation of calibration equation through multiple linear regression

The calibration equation from the multiple linear regression analysis was based on second derivative spectra and K values, or percentages of IMP and HxR determined by chemical analysis, and the results are shown in Table 2. For the two wavelengths selected, multiple correlation coefficients of over 0.8 were obtained using each of the three chemical values, and high correlations of over 0.9 were obtained using K values for calibration. In a comparison of two sample sets, Nos. 1 and 2, set No. 1 was determined to be more suitable as a calibration set because it had a wider distribution of K values than set No. 2, as shown in Figure 2 (1A – 2A). On the other hand, results with higher multiple correlation coefficients obtained by using the three wavelengths selected for calibration weren't adopted in this study in order to avoid over-characterization of the calibration sets. Consequently, the best result in terms of the calibration equation was thought to be obtained using the K values of Sample Set No. 1 at two wavelengths (713 and 1,181 nm). The relationship between wavelength and multiple correlation coefficients in Sample Set No. 1 is shown in Figure 3. The correlation coefficient was 0.901, the standard error of calibration (SEC) was 4.61%, and the calibration equation can be shown by the following formula:

$$\text{K value (\%)} = 22.1 + 2546.9\ d^2\log(1/R_{713}) - 161.6\ d^2\log(1/R_{1181})$$

The results of validation for determining K values, or percentages of IMP and HxR, using three sets of pork samples (Nos. 1 – 3) in turn for calibration and validation, are shown in Table 3. The validation result using calibration set No. 1 (n = 76) and the K value resulted in a multiple correlation coefficient (R) of 0.820 (Bias = 5.500, SEP = 4.04) when Sample Set No. 3 (n = 46) was used as a validation set, while R was 0.635 (Bias = −8.850, SEP = 5.31) when set No. 2 (n = 41) was used. Regarding the distribution of K values in Sample Set Nos. 2 and 3, both the minimum and average values were observed to be lower in set No. 3, as shown in Table 1. These results suggest that the calibration equation for detecting the K values shown above might be more applicable to pork Boston butt that has a low K value of less than 40%.

Conclusion

In this study, we obtained a calibration equation for the prediction of K values of pork Boston butt using NIR spectroscopy. In the multiple linear regression analysis, the K value was the most suitable of all the values investigated in the study for use as an objective variable. However, the

Table 2. Results of calibration for determining K, IMP, HxR and Hx values (%)

Sample sets	Objective variable	Wavelength selected (nm) λ_1	Wavelength selected (nm) λ_2	R*1	SEC*2 (%)	Calibration equation
No. 1 (n = 76)	K value (%)	713	1181	0.901	4.61	$Y = 22.1 + 2546.9\ d^2\log(1/R_{713}) - 161.6\ d^2\log(1/R_{1181})$
	IMP (%)*3	715	765	0.882	4.76	$Y = 68.9 + 2363.0\ d^2\log(1/R_{715}) - 1206.0\ d^2\log(1/R_{765})$
	HxR (%)*3	714	1180	0.889	4.15	$Y = 17.9 + 2156.4\ d^2\log(1/R_{714}) - 153.9\ d^2\log(1/R_{1180})$
No. 2 (n = 41)	K value (%)	761	1204	0.906	3.16	$Y = 15.3 + 2502.5\ d^2\log(1/R_{761}) - 386.3\ d^2\log(1/R_{1204})$
	IMP (%)	761	1204	0.813	4.24	$Y = 85.1 - 2163.4\ d^2\log(1/R_{761}) - 419.2\ d^2\log(1/R_{1204})$
	HxR (%)	761	1204	0.898	2.90	$Y = 9.1 + 2182.3\ d^2\log(1/R_{761}) - 360.5\ d^2\log(1/R_{1204})$
No. 3 (n = 46)	K value (%)	713	958	0.860	5.03	$Y = -41.9 + 1855.4\ d^2\log(1/R_{713}) + 2129.3\ d^2\log(1/R_{958})$
	IMP (%)	713	1167	0.849	4.65	$Y = 204.7 - 1773.8\ d^2\log(1/R_{713}) + 150.3\ d^2\log(1/R_{1163})$
	HxR (%)	714	958	0.872	4.10	$Y = -35.8 + 1664.8\ d^2\log(1/R_{714}) + 1832.6\ d^2\log(1/R_{958})$

*1 R : multiple correlation coefficient.

*2 SEC : standard error of calibration.

*3 IMP(%), HxR(%) : percentages of IMP and HxR in whole ATP-related compounds.

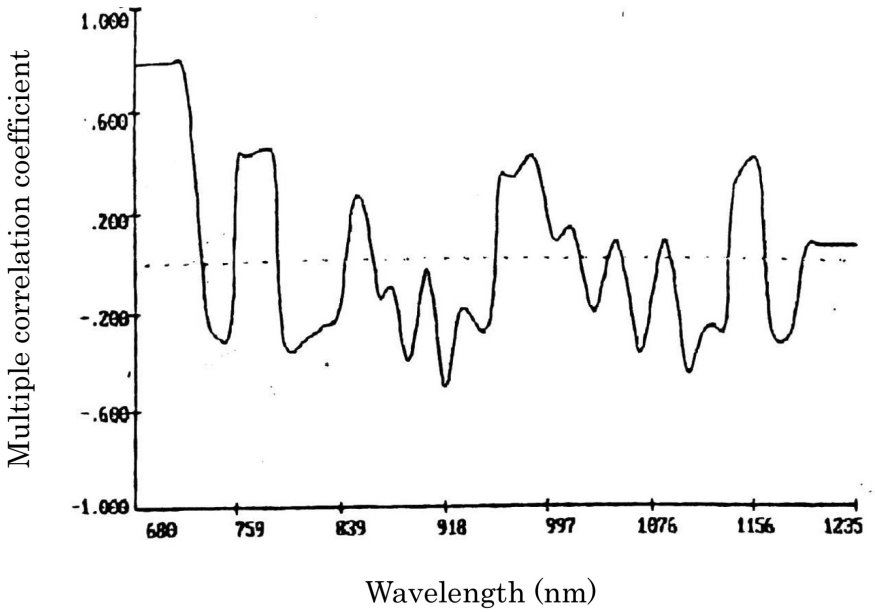

Fig.3　Relationship between wavelength and the multiple correlation coefficient of the second derivative spectra at each wavelength of Sample Set No. 1.

Table 3.　Results of validation to determine K, IMP, HxR and Hx values (%)

Calibration set	Objective variable	Wavelength selected (nm) λ_1	λ_2	Validation set	R*1	Bias*2	SEP*3 (%)
No. 1 (n = 76)	K value (%)	713	1181	No. 2	0.635	- 8.850	5.31
				No. 3	0.820	5.500	4.04
	IMP (%)*3	715	765	No. 2	0.729	1.110	3.05
				No. 3	0.837	- 9.390	3.74
	HxR (%)*3	714	1180	No. 2	0.614	- 9.410	4.94
				No. 3	0.808	3.900	3.55
No. 2 (n = 41)	K value (%)	761	1204	No. 1	0.381	0.349	2.62
				No. 3	0.696	5.640	2.50
	IMP (%)	761	1204	No. 1	0.399	1.100	2.85
				No. 3	0.680	- 4.200	2.38
	HxR (%)	761	1204	No. 1	0.452	0.808	2.38
				No. 3	0.707	5.420	2.05
No. 3 (n = 46)	K value (%)	713	958	No. 1	0.830	- 9.400	3.15
				No. 2	0.374	- 12.900	4.29
	IMP (%)	713	1167	No. 1	0.756	6.330	3.29
				No. 2	0.597	11.500	4.65
	HxR (%)	714	958	No. 1	0.775	- 8.040	2.84
				No. 2	0.319	- 11.700	3.79

*1 R：multiple correlation coefficient.

*2 Bias：mean difference between actual and NIR predicted values.

*3 SEP：bias-corrected standard error of prediction.

content of ATP, ADP and AMP in pork Boston butt was already quite small 24 hours after slaughter. This result suggests that mK values[6], which can be calculated by the following formula, might be more suitable than K values as an objective variable in the analysis:

$$mK\ (\%)^{6)} = (HxR+Hx) / (IMP+HxR+Hx) \times 100$$

Finally, in regard to pork quality in terms of total palatability, Okumura *et al.*[1] reported that the quality of pork stored for 20 days was best when it was stored under vacuum packing at 4℃ for 2 to 30 days. It was also reported that the most common substance among all ATP-related compounds was HxR, with increasing Hx in pork stored for 20 days.[1] These results suggest that not only K values and mK values but also HxR (%) and Hx (%) might be suitable as objective variables for estimating pork quality in terms of total palatability.

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〈研究ノート〉

近赤外分光法による豚肉の鮮度判定恒数K値の非破壊分析

Non-destructive determination of K values in pork using near infrared spectroscopy

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中山　洋[5]，鈴木　敏博[6]，堀内　篤[7]

要　旨

畜肉は、と殺後の一定期間の熟成を経て、食用とされる。その期間は畜種と様々な要因により異なるが、５℃に貯蔵した場合、豚肉では４～６日間とされる。豚肉は死後硬直を経て、自己消化により肉質がやわらかくなり、うまみや香りを増して熟成し、はじめて風味のある食肉になる。つまり豚肉においては、肉が新鮮であることが、おいしさを主要因とした場合の肉の品質に直結するわけではない。豚肉にとって鮮度とは、その自己消化や熟成の進行程度とも言い換えることができる。

豚肉の自己消化の程度は、と殺後の経過時間や保存状態に大きく影響される。そのため、様々な状態の豚肉が流通することになり、もとは同一の豚肉であっても消費者に異なる評価をされる可能性がある。流通現場において、あるいは消費者として、目の前の肉の貯蔵履歴や、それら要因によりもたらされた肉の状態を知ることは不可能に近く、目の前の豚肉の保存期間や熟成の程度を、簡便に推定できるのであれば、肉質評価の上で参考になるものと思われる。

そこで本研究では、簡便に豚肉鮮度を評価する手法の開発を目的として、近赤外分光法により、非破壊的に、鮮度恒数K値を分析する方法を検討した。まず、豚３頭から採取した肩ロース肉を細かく切り分けたものを、４℃で様々な期間貯蔵することにより、幅広いK値の分布幅を持つ試料群を作成し、その試料群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値を求めた。そして、それらの結果を、装置付属の解析ソフトを用いて重回帰分析することにより、豚肉の鮮度推定式として、目的変数にK値、二次微分スペクトルの波長としては、713 および 1181 nmが選択された、以下に示す較正方程式を得た。

$$K値\ (\%) = 22.1 + 2546.9\ d^2\log(1/R_{713}) - 161.6\ d^2\log(1/R_{1181})$$

この方程式を別個体由来の２つの試料群で評価したところ、K値の平均値が低く、値の分布も低いものから幅広い試料群との間に、より高い精度が認められた。このことから、この方程式はK値40%以下の試料に、より適用される可能性が考えられた。

また本研究では、鮮度指標としてK値を採用したが、と殺後24時間経過した豚肉試料においては、K値の計算式：

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

におけるATP、ADPおよびAMPが殆ど存在しないことが、本実験でも確認された。そのため、既に報告されている修正K値（mK値）：

$$mK値\ (\%) = (HxR+Hx)/(IMP+HxR+Hx) \times 100$$

をK値のかわりに豚肉の鮮度判定恒数として解析に利用することにより、より精度の高い較正方程式が得られる可能性も推察された。

さらに、豚肉の品質を総合的なおいしさから評価する観点から見ると、真空包装した豚肉を４℃で30日間保存した研究においては、と殺後20日目の豚肉が官能的に最も優れていたとの報告があり、そして、この時点での肉試料中のATP関連物質としてはHxRが最も多く、Hxも増加途上にあったことから、豚肉の総合的なおいしさを非破壊的に分析する上では、K値やmK値以外にも、HxRやHxの含有量が解析上の目的変数として有効である可能性が推察された。

キーワード：近赤外分光法，非破壊，豚肉，鮮度，K値，核酸関連物質