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Establishment of Quality Evaluation Sensor for Pork Fillets Freshness during Storage based on *Ki* value Analysis

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Ki value 분석을 활용한 보관 중 돼지고기 안심의 신선도 품질평가 센서 구축

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Food safety is a fundamental requirement for consumers. Therefore, this study was aimed to determine the freshness of pork fillets based on freshness index (*Ki* value) analysis during storage. This study obtained a total of 24 pork fillets. This study was divided into 4℃ and -20℃ temperatures. Again, each temperature was divided into 4 different groups based on storage durations (15d). Each group was obtained in triplicate. The level of malondialdehyde (MDA) value was significantly increased at 4℃ than the -20℃ storage with the duration of storage days. The levels of inosine-5'-phosphate (IMP) in pork fillets were significantly decreased at 4℃ and -20℃. Hypoxanthine (Hx) levels were significantly increased at 15 days than those at 0 days during storage at both temperatures (4℃ and -20℃). Subsequent freezing increased more inosine levels at -20℃ than storage at 4℃. The *Ki* values were increased during storage days at both temperatures, although after 15d storage, its values at 4℃ were significantly higher than those at -20℃. These results demonstrated that storage period and temperature might affect the freshness of pork fillets via lipid oxidation and nucleotide degradation. This study suggested that the *Ki* value will be the crucial indicator of measurement of freshness of stored pork fillets.

Key words : *freshness, Ki value, nucleotide degradation, pork fillet, storage*

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Ⅰ. Introduction

Food quality is an important topic to meat industries and consumers (Silvestri et al., 2020). Meat is a source of protein, and food with good sensory properties, of which taste and flavor are the most important (Vani et al., 2006). Pork is a crucial source of food, which contributes to full fill protein requirement. However, the pork industry is facing difficulties to determine the freshness of pork after storage. The pork quality is determining by various factors, including freshness, color, firmness, and exudation (Lebret and Čandek-Potokar, 2021). Freezing temperature can inhibit undesirable biochemical reactions such as lipid oxidation (Al-jasser, 2012). During storage, oxidation generally occurs in the lipid fraction of meat. It is a major cause of changes in meat quality parameters. Lipid peroxidation is primarily responsible for meat and meat product quality deterioration (Min et al., 2008).

Nucleotide degradation was regarded as a contributor to the quality and freshness of meat and meat products (Feng et al., 2016). Total adenosine triphosphate (ATP) concentration and related compounds in muscles as well as patterns and rates of changes in their levels during storage are species dependent and muscle dependent. It has been generally accepted that ATP depletion is the genuine cause of the onset of rigor mortis (Alvarez et al., 2019). After death, decomposition of ATP in meat have produced adenosine diphosphate (ADP), adenosine-5'-phosphate (AMP), inosine-5'-phosphate (IMP), and other relative compounds. During storage, many methods have been used to assess fish and meat quality. Among these methods, the *Ki* value was usually used as the most effective indicator for the freshness of fish. A previous study reported that the *Ki* value is widely used to measurement of fish quality (Watanabe et al., 2005). Moreover, another study demonstrated that *Ki* value to evaluate the quality of rainbow trout fillets at super chilling (-3℃) and chilled (3℃) storage (Shen et al., 2015).

Food safety is a legal and fundamental requirement for consumers. All food products offered for sale must be safe (Dominic, 2002). Many researchers have been regularly searching for improved methods to preserve or extend the shelf life and safety of various food and food products (Chang et al., 1998). Recently, increases the food and food products' self-life based on consumer demands. Therefore, we tried to establish a technology that identified pork meat freshness very easily. Based on the above circumstance, the aim of this study was to investigate the pork fillets' freshness during different storage temperatures and times by *Ki* value analysis.

Ⅱ. Materials and Methods

1. Ethics statement

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Jeonbuk National University (CBNU 2018-097), Republic of Korea. Animal care and handling are in compliance with the regulations of the IAEC Guidelines for the Euthanasia of Animals: 2015. The sampling procedures complied with the "Guidelines on Ethical Treatment of Experimental Animals".

2. Sampling and experimental design

The fresh pork meat was provided by Doozy Pork (South Korea) and immediately transported to the laboratory. Pigs were Yorkshire × Landrace × Duroc (YLD) three-way crossbred male with an average age of 175 days old. The commercial feed was provided according to the regimens of FARMSCO Inc. Total of 24 samples were collected from randomly 3 different pigs all pigs were the same batch. This experiment was categorized by 4℃ and -20℃ temperature, and each temperature was divided into 5 subgroups based on pork storage duration (0, 5, 10, and 15 days). Total 3 samples were obtained for each group (triplication).

3. Chemicals and reagents

Thiobarbituric acid (TBA, 99%), 1,1,3,3-tetramethoxypropane (TMP, 99%), butylated hydroxy-anisole (BHT, 98.5%), methanol (99.8%), hypoxanthine (Hx), inosine (HxR), IMP, and AMP were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid (> 99.5%) was purchased from Daejung (Daejung, Korea). All chemicals and reagents were of analytical grade.

4. Extraction of malondialdehyde (MDA) in meat samples and calibration standards

The published procedure was followed to determine MDA values (Zeb and Ullah, 2016). Briefly, one gram of blended meat sample was taken in 15 mL test tube and added 5 mL of 50% glacial acetic acid in water (AW). Then 50 μL of BHT (0.01%) was added to prevent further oxidation. Samples were shaken (Laboshaker R100, Labogene, Daejeon, South Korea) at

high speed (60 rpm) for 1 h and centrifuged by 3000 × g for 10 min at 40℃. The filtrate was centrifuged when required, and was used for analyses. The standard stock solution of TMP (1 mM) was prepared in glacial acetic acid. From the stock solution, different concentrations (0.1, 0.2, 0.4, 0.6, and 0.8 μM) were prepared. The calibration curve was constructed in the concentration range of 0.1 to 1.0 μM. The standard TMP solution (1 mL) was added into a 10 mL test tube and mixed with 1 mL TBA (4.0 mM in 50% acetic acid). The mixture was heated in a water bath at 95℃ for 60 min. After cooling at room temperature, absorbance was measured at 532 nm using a UV spectrophotometer (Multiskan Go, Thermo Scientific, USA) and a calibration curve for the standard was prepared. A linear regression curve of standard TMP at concentration of 0.1-1.0 μM with a regression equation of $y = 0.0441x + 0.0004$ and correlation coefficient of 0.9995. Each point in the regression curve represents replicated measurement ($n = 3$). A blank sample was used for both the standard and the sample. The extract of each sample (1 mL) was mixed with 1 mL TBA reagent and the above procedure was repeated. Thiobarbituric acid reactive substances (TBARS) was calculated as μM/g of the sample using the following formula 1.

$$MDA\ (\mu M/g) = \frac{Ac \times V}{W} \quad (1)$$

Where Ac is the amount determined from the calibration curve, W is the weight of the sample taken, and V is the volume in mL or dilution factor of the total extract prepared. The unit was then converted into mg MDA/kg of meat.

5. Measurement of nucleotide contents

To measure nucleotide contents, meat samples (5 g) were mixed with 20 ml of distilled water (DW) and homogenized for 30 sec twice. Then 25 mL DW was added, mixed, and centrifuged by 3000 rpm for 20 min at 4℃. Then 5 mL supernatant was transferred to a test tube and 10 mL of 0.5 M perchloric acid was added in to the tube. After holding at room temperature for 15 min, the solution was filtered with Whatman No.4 paper to extract nucleic acids. Extracted nucleic acids (1 mL) were transferred into 2 mL tube and filtered with a syringe filter (HLB-M, 0.45 μm particle size, 13 mm, Futecs Co., Ltd.). The filtrate was then analyzed using HPLC (Shiseido Nanospace SI-2, Shiseido Co., Ltd. To-kyo, Japan) equipped with a Imtakt Cadenza CD-C18 reverse phased column (4.6 × 250 mm, 3 μm, Imtakt Corp., USA). Two mobile phases

were used: A) 1000 mL distilled water + 5 mL of 40% TBA-OH (tert-butyl ammonium hydroxide) + 1 mL of 85% H_3PO_4 (phosphoric acid), and B) 1000 mL 80% methanol + 5 mL of 40% TBA-OH + 1 mL of 85% H_3PO_4. The flow rate of the mobile phase was set at 0.8 mL/min and the injection volume was 10 μL. The column temperature was maintained at 40℃ and the detection was monitored at a wavelength of 250 nm. Peaks of individual nucleotides were identified using retention times for standards: Hx, HxR, IMP, and AMP. The concentration of nucleotide was calculated using the area for each peak.

6. Determinations of *Ki* values

Amounts of nucleotides related compounds were determined and calculated based on standard IMP, HxR, and Hx. Previous study calculates the *Ki* value as an indicator of freshness (Karube et al., 1984). The formula of *Ki* value calculation is below.

$$Ki\,value = \frac{HxR + Hx}{IMP + HxR + Hx} \times 100 \quad (2)$$

Where *HxR*, *Hx*, and *IMP* are the amount of inosine, hypoxanthine, and inosine-5'-phosphate, respectively. The *Ki* value is an important indicator of freshness. And the lower *Ki* value means the fresher meat.

7. Statistical analysis

All statistical analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC, USA). The data of each day of pork storage of both temperatures were analyzed using Duncan's multiple range test followed by the analysis of variance (ANOVA). The student's t-test was used to analyze the statistical differences between 4℃ and -20℃ at different storage periods. Values are expressed as mean ± SD. Statistical significance was considered at $p < 0.05$.

Ⅲ. Results

1. MDA analysis

Results of lipid oxidation in pork meat during storage at 4℃ and -20℃ are shown in Table 1. In 4℃, the amounts of MDA were significantly increased in pork fillets at 10 and 15 days storage than 0 and 5 days. In contrast, at the -20℃, the amount of MDA was not significantly changed during storage among different days ($p < 0.05$). However, the MDA values were significantly increased ($p < 0.05$) in -20℃ than 4℃ at 5, 10, and 15 days of storage period.

Table 1. Changes in malondialdehyde (MDA) values of pork fillets during different storage period at 4℃ and -20℃

Parameters	Storage temperature	Storage period (day)			
		0	5	10	15
MDA (mgMDA/kg)	4℃	0.51 ± 0.07^{b}	$0.83\pm0.18^{ab,*}$	$0.94\pm0.20^{a,*}$	$0.95\pm0.15^{a,*}$
	-20℃	0.51±0.07	0.49±0.11	0.49±0.20	0.53±0.19

Note: Mean values are presented at mean ± SD

$^{a-b}$ Means with different letters are significantly different among 4℃ groups ($p < 0.05$)

* Significant difference between 4 and -20℃ at same day based on t-test ($p < 0.05$)

2. Nucleotide related compounds analysis

Nucleotide degradation results are shown in Table 2. The IMP was significantly ($p < 0.05$) degraded with the duration of storage time at both 4℃ and -20℃. Although, the amount of IMP degradation was not significantly different in between 4℃ and -20℃.

The concentration of HxR in pork fillets stored at 4℃ reached its peak level at 0.20 μM/g on day 15. The HxR content in pork fillets stored at -20℃ showed a similar trend. However, it was increased faster, reaching its peak level at 0.25 μM/g on day 15, it was not significantly changed. Hx level was increased during the storage of meat at 4℃, resulting in higher value at day 15 which was significantly ($p < 0.05$) higher than that at day 0. Similarly, subsequent freezing increased the Hx level at -20℃ with the higher value at day 15 which was significantly higher than day 0. There were no significant differences in Hx level between the two storage temperatures during all storage periods, although the Hx level was decreased more at -20℃ than that at 4℃.

Table 2. Post-mortem nucleotide catabolites of pork fillets during different storage periods at 4 and -20℃

Parameters	Storage temperature	Storage period (day)			
		0	5	10	15
IMP	4℃	2.19 ± 0.02^{A}	1.09 ± 0.07^{B}	0.56 ± 0.18^{C}	0.16 ± 0.15^{D}
	-20℃	2.19 ± 0.09^{a}	1.13 ± 0.03^{b}	0.65 ± 0.19^{c}	0.26 ± 0.13^{d}
HxR	4℃	0.13±0.04	0.17±0.08	0.18±0.05	0.20±0.14
	-20℃	0.13±0.05	0.17±0.09	0.20±0.11	0.25±0.16
Hx	4℃	0.10 ± 0.03^{B}	0.19 ± 0.04^{AB}	0.18 ± 0.10^{AB}	0.30 ± 0.19^{A}
	-20℃	0.10 ± 0.02^{b}	0.11 ± 0.05^{ab}	0.13 ± 0.06^{ab}	0.22 ± 0.17^{a}

Note: $^{A-D}$ Means with different letters are significantly different among 4℃ groups ($p<0.05$)
$^{a-d}$ Means with different letters are significantly different among -20℃ groups ($p<0.05$)
HxR: inosine, Hx: hypoxanthine

3. *Ki* value analysis

Results of variations in *Ki* value of pork fillets during storage are presented in Fig. 1. *Ki* values during storage at both temperatures were increased with duration storage time. The *Ki* values in pork fillets stored at 4℃ were increased more than those at -20℃ in consecutive storage period without significant differences. However, the *Ki* value at 4℃ was significantly ($p < 0.05$) increased compared to -20℃ at 15 days of storage.

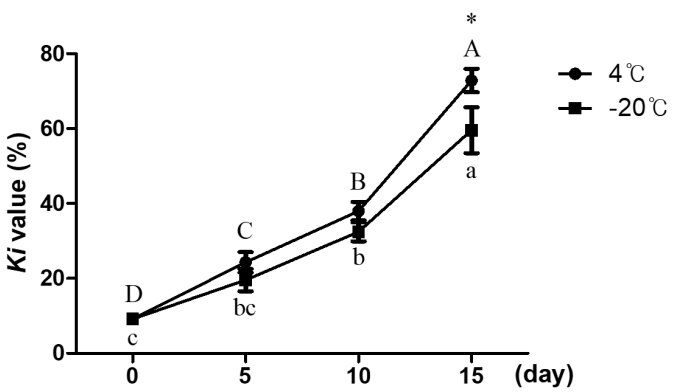

Fig. 1. Changes in *Ki* values of pork fillets during different storage period.

$^{A-D}$ Means with different letters are significantly different among 4℃ groups ($p < 0.05$).
$^{a-c}$ Means with different letters are significantly different among -20℃ groups ($p < 0.05$).
* Significant difference between 4 and -20℃ at same day based on t-test ($p < 0.05$).

Ⅳ. Discussion

In this study, established a method of investigating the freshness of pork fillets by using *Ki* value analysis. The freshness and quality of meat depend on many factors, including the meat storage temperature and duration (Mezemir et al., 2019). It has been reported that chicken meat at 6 days after storage at 4℃ has significantly higher TBARS value than that at day 0 (Al-jasser, 2012). Besides, Jin et al. (2005) reported that the TBARS values of fermented pork increase significantly during storage. TBARS value has been commonly used to indicate the degree of lipid oxidation in meat (beef) and meat products (Grotta et al., 2017). A previous study reported that low MDA content of muscles inhibited muscle lipid peroxidation and improved the meat quality by reducing the rate of water loss and maintaining the cell membranes' integrity (Li and Chen, 2009; Liu et al., 2013). It has been reported that subsequent freezing can result in significantly higher TBARS values of meat at 90 days after storage (Popova et al., 2009). Previous studies demonstrated that lipid oxidation of meat is mostly affected by storage duration and temperature (Sun et al., 2002; Smet et al., 2005). Low TBARS values in meat indicate better quality. However, the chilled and frozen conditions reduce the lipid oxidation in meat (Zarzycky and Swiniarska, 1993). Although, the lipid oxidation does not discontinue or stabilize at low temperatures. The oxidation is responsible for the degradation of sensory characteristics of food while lipids are susceptible to pro-oxidant factors. It has been reported that once the oxidation process was started, it cannot be stopped, thus lowering the shelf life of food (Rahman et al., 2015).

The nucleotides compound is degraded by different enzymatic reactions that decline meat flavor and color (Li et al., 2020). Generally, after the death of animals, started producing hypoxanthine from ATP through a mechanism (Fig. 2). It has been reported that the IMP is slowly degraded to HxR and Hx, resulting in loss of fish flavor (Itoh and Kimura, 2002). The IMP is the major nucleotide in the muscle and is responsible for good desirable flavors (Vani et al., 2006). A previous study stated that the IMP has been directly correlated with the flavor and freshness in fish (Duan et al., 2020). Therefore, the IMP has been considered a deleterious substance that affects meat taste (Saito et al., 2007) and is also associated with favorable flavor (Tikk et al., 2006). The appropriate storage condition prevents the meat spoiled. Therefore, the accumulation of HxR was might be delayed at -20℃ compared to that at 4℃. Our results showed that increased frozen temperature can prevent the accumulation of HxR than 4°C for storage. Our result is consistent with the previous study, where the rainbow trout fillets were stored in super chilling (-3℃) and chilled (3℃) temperature (Shen et al., 2015). The most

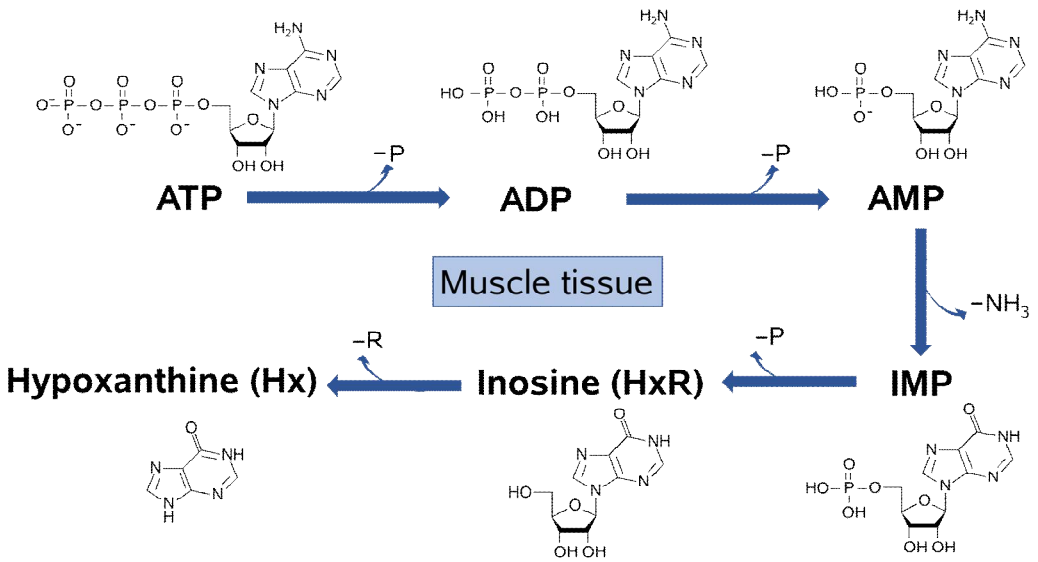

Fig. 2. ATP degradation pathway to Hx following arrest of cellular respiration post-mortem in muscle tissue. Changes in *Ki* values of pork fillets during different storage period.

important taste compound in meat is Hx which is responsible for bitterness. The bitter taste is done by hypoxanthine while inosinic and glutamic acid have a synergistic effects on umami taste (Jo et al., 2012). Our results demonstrated that -20℃ could prevent the accumulation of Hx more effectively than 4℃ for storage.

Generally, the *Ki* value is calculated from ATP degradation of fish in after death condition. Therefore, the *Ki* value is recommended as an important indicator of fish freshness (Özogul et al., 2006). Our results demonstrated that the *Ki* value at -20℃ was lower than that at 4℃ during storage. The lower *Ki* value indicates fresher meat. However, the *Ki* value 10% higher than the normal value as considered normal in fish (Khodabux et al., 2007). In our results, the *Ki* value was similar for the two temperatures due to the large accumulation of HxR and Hx at the different duration of storage of pork fillets. However, the *Ki* value showed significantly difference between two different temperatures at 15 days of storage. Interestingly, previous study reported that *Ki* values could use as an indicator of the freshness of chicken and beef (Saito et al., 2007).

Ⅴ. Summary

The *Ki* value has been recognized as the most effective and objective index for the freshness of fish. This study showed that the lipid oxidation process in aged pork fillets was influenced by storage temperature. Duration of storage significantly affected the formation of MDA. In addition, MDA values of pork fillets were increased more at 4℃ than those at -20℃. Among the IMP, Hx content and *Ki* values were a better freshness indicator for pork fillets during -20℃ storage than the 4℃ storage period. Based on MDA and *Ki* values, lipid oxidation and nucleotide degradation occurred more at 4℃ than those at -20℃ during the storage of pork fillets, thus affecting their freshness. The lower *Ki* value indicated the fresher pork fillets. Today's pork industry is highly competitive. The pork industry would prefer and give most attention to produce high quality meat for the satisfaction of consumers. The storage temperature and duration are directly associated with the meat quality factors like freshness, taste, flavor, and healthfulness. This study will be helpful to improve a method to measure the pork quality during storage in different temperatures and duration.

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