Development of a freshness indicator for monitoring the quality of beef during storage

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Abstract Quality of beef and detect changes in freshness during storage was evaluated using freshness indicator. The freshness indicator can indicate spoilage or freshness of the packaged product through color changes directly detected by the naked eye. We also monitored changes in pH, 2-thiobarbituric acid reactive substances, volatile basic nitrogen, and total bacterial count. A change in the color of the indicator reflects the pH of the headspace of the beef packaging. Beef was stored at 20 °C for 24 h, after which the color of the freshness indicator changed. Results of color change from freshness indicator are similar to quality parameters including chemical, microbiological, and sensory evaluation that are commonly used to characterize the freshness of beef. The results show that this freshness indicator is useful for determining the shelf life of beef products by monitoring physicochemical changes and microbial safety.

Keywords Freshness indicator · Volatile basic nitrogen · Food quality · Package · Beef

Introduction

As household income has increased and eating habits have become more westernized, the consumption of fresh meat has increased as well (Kim et al., 2012b). The food industry has striven to develop new technologies that enable consumer expectations regarding the safety and sensory quality of food to be met (Vaikousi et al., 2009), as well as pursuing the production of safe livestock products, the improvement of distribution systems to maintain freshness, and the development of quality and safety assessment technology. Despite these efforts, there is still no reliable way to scientifically confirm the freshness of consumer-grade meat except for checking its color, smell, and/or expiration date (Shin et al., 2006). The chemical composition of meat is very complex, consisting of about 1–5% sugars, 10–20% protein, and 10–30% fat. Therefore, the decomposition of meat is faster because microorganism contamination is difficult to control (Kuswandi et al., 2015).

The freshness of refrigerated meat is reduced by biochemical, physicochemical, and microbiological changes. This loss of freshness indicates that the meat has started to decay. Microorganisms can act on proteins via proteolytic activity and transform them into smaller compounds, such as free amino acids. These amino acids undergo oxidative deamination, decarboxylation, and desulfurization, resulting in NH₃, CO₂, and H₂S. In addition to these, another indicator of the microbial decay of proteins in food is high levels of highly total volatile basic nitrogen (TVBN), such as ammonia, dimethylamine (DMA), and trimethylamine (TMA). Changes in the color of pH dyes (e.g. bromocresol green, methyl yellow, methyl red, and phenol red) can be used to detect acidic or basic volatile compounds, which

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exhibit irreversible changes in non-visual appearance (Rukchon et al., 2014).

Three types of criterion are usually employed to determine beef quality: sensory, microbiological, and chemical. However, these methods have a number of disadvantages, including time-consuming detection processes, low accuracy, and/or high costs. Therefore, in order to evaluate the quality of meat in the field, such as at home, in supermarkets, or at distribution centers, a simple, low-cost, and highly efficient method is necessary. This would allow consumers to directly determine the freshness of meat (Kuswandi and Nurfawaidi, 2017). Intelligent packaging can communicate with consumers or food manufacturers and can detect internal or external environments in the package to provide early warning to users. One type of intelligent package, the freshness indicator, is a small device that is printed in the form of packaging film or package labels (Chen et al., 2018). Freshness indicators provide information about product quality based on chemical changes or microbial growth in the food product. Especially, Color-based pH-sensitivity indicators have been reported as promising intelligent packaging for the determination of microbial metabolites for packaged fish, shrimp, broiler chicken freshness (Lee et al., 2019). These indicators are natural dyes of bromocresol green, curcumin, bromocresol purple, which are changed from the acid form to the basic form due to the increase in the pH of the headspace caused by volatile amines which are generated when meat and aquatic products spoilage (Ezati et al., 2019). So, pH-sensitive dyes can be used to develop sensors for the detection of basic volatile amines in meat, fish, and poultry. One indicator of the microbial degradation of food proteins is VBN (Biji et al., 2015). It has been reported that the increase in VBN during the storage of meat is due to the decomposition of muscle proteins into amino acids by the enzymes secreted by microorganisms. In addition, VBN is seen as an indicator of odor generation in beef, which shows a tendency of change among several quality factors and temperature dependency similar to the occurrence of offensive order.

Packaging technology that incorporates indicator sensors to monitor food spoilage has been proven useful in ensuring the quality and safety of packaged foods. It also has the advantages of being able to be quickly applied on-site at a low cost without requiring expensive equipment and of allowing the management of food quality in real time (Shukla et al., 2015).

The aim of this study is to evaluate the relationship between the amount of volatile compounds and the number of microorganisms and to develop display a clear

change in color of indicator when beef has lost its freshness.

Materials and methods

Materials and chemicals

Beef was obtained from a local market in Gyeonggi-do, Korea. Cellulose acetate (mw ~ 30,000 g/mol), 2-butanone, and sodium hydroxide were obtained from Junsei Chemical Co. (Tokyo, Japan). Methyl red solution was purchased from Samchun Chemical Co. (Seoul, Korea), while dibutyl phthalate (DBP) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetone was purchased from Burdick and Jackson Labs (Muskegon, MI, USA), and filter papers were obtained from Whatman GE Healthcare and Life Sciences (Seoul, Korea). Polytetrafluoroethylene (PTFE) was purchased from Hyundai Micro., Ltd. (Seoul, Korea). All other chemical reagents were analytical grade and obtained from Burdick and Jackson.

Freshness indicator development

Freshness indicator sensor spots were produced with a coating of a methyl red-polymer mixture. A total of 0.6 g of cellulose acetate was placed in 20 mL of a 1:1 mixture of acetone and butanone. After it had been completely dissolved using ultrasonication (JAC 2010, Korea Chemical Research Institute), 0.62 g of dibutyl phthalate was added (Byrne et al., 2002). 15 mg of methyl red was added to the polymer matrix solution. Freshness indicator sensors were produced by dispensing 20 μ L of the polymer matrix solution onto a paper disc with an automatic micropipette. The freshness indicator was then inserted between PET film and PTFE, which acted as a hydrophobic gas permeable membrane that passed gaseous compounds while protecting the freshness indicator from moisture (Pacquit et al., 2007). A prototype freshness indicator is presented in Fig. 1.

Fig. 1 Prototype sensor for freshness indicator

pH measurement

The pH of the beef samples was measured using a pH meter (Orion 3-star Meter; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at storage temperature (20 °C). For this measurement, 5 g of minced beef was mixed with 20 mL of distilled water. The pH meter was calibrated using pH buffer solutions (10.00 ± 0.01 , 7.00 ± 0.01 and 4.01 ± 0.01 at 25 °C). All measurements were conducted in triplicate.

Measurement of volatile basic nitrogen content

VBN content was determined using the Conway micro-diffusion technique from Kim and Shin (2011). 5 g of beef samples were placed in a 50-mL tube and 25 mL of distilled water was added. The samples were homogenized at $25,000 \times g$ for 30 s, adjusted back to 25 mL with distilled water, and left for 30 min. The homogenate was subsequently filtered using filter paper (Whatman No. 1). Following this, 1 mL of the filtrate was added to the external space of a Conway dish and 1 mL of 0.01 N H_2SO_4 was added to the internal space. The Conway dish was then sealed immediately after adding 1 mL of saturated K_2CO_3 to the external space. The sealed Conway dishes were gently shaken and incubated at 25 °C for 60 min. After this, 10 μ L of Branswik indicator solution was dropped into the inner space and 0.01 N NaOH was added for titration. The calculation for VBN levels was as follows:

$$\text{VBN (mg}\%) = \frac{0.14 \times (b - a) \times f}{S} \times 100 \times d$$

where S is the weight of the meat sample (g), b is the volume of NaOH added (mL), a is the volume of NaOH added to the sample (mL), d is the factor of dilution, and f is the standard factor of NaOH (Min et al., 2007).

Microbial analysis of the beef samples

Under sterile conditions, approximately 10 g of minced beef was mixed with 90 mL of sterile 0.1% peptone water (Difco; Becton, Dickinson company) in stomacher bags. This mixture was homogenized for 4 min at room temperature. The sample was serially diluted to 10^{-7} . Petrifilm aerobic count plates were inoculated with 1 mL of each sample dilution. After inoculation, the samples were incubated for 48 ± 2 h at 35 ± 1 °C. Total bacterial counts (TBC) were recorded for all colonies dyed in various shades of red (Wohlsen et al., 2006).

Measurement of 2-thiobarbituric acid reactive substance levels

The 2-thiobarbituric acid reactive substance (TBARS) levels in the meat were determined using the Food TBARS Assay Kit (Oxford Biochemical Research, Inc., MI, USA) according to the protocol described by the manufacturer (Park et al., 2017).

Chromaticity analysis

The chromaticity of the sensors was measured by placing a portable colorimeter (CR-300, Minolta, Tokyo, Japan) in each sensor for 72 h. To evaluate the applicability of the developed freshness indicator to monitor the freshness of meat, the sensor was attached to the top of the beef sample package with a hole and stored at 20 °C in direct contact with the air inside the package. The sensor under measurement was placed in white in a closed box to avoid light interference from the surrounding environment (Kim et al., 2012a). The Hunter color scales a (redness), b (yellowness), and L (lightness) were measured in triplicate following the manual after 0 h, 12 h, 18 h, 24 h, 36 h, 48 h, 60 h, 72 h of storage. The ΔE value was calculated using the following equation:

$$\Delta E = \sqrt{(L - L')^2 + (a - a')^2 + (b - b')^2}$$

HS-SPME analysis

The meat samples were cut into accurately weighed pieces of log, homogenized, and placed into 50-mL plastic centrifuge tubes. Following this, 20 mL of 0.5 N HCl was added to each tube, vortexed for 5 min, and then centrifuged at 4000 rpm for 10 min at 5 °C. The supernatant was poured into a 50-mL volumetric flask and made up to the mark. Magnetic stirrer bars and 5.0 μ g mL^{-1} internal standard were put into a 20-mL SPME vial, followed by a 0.5 mL aliquot and 1 mL of 15 M NaOH solution. The vial mixture was stirred for 15 min and then the SPME needle guide was inserted for headspace extraction. The position of the fibers and the rotation speed of the stirrers were carefully adjusted to prevent spillage from the fibers. Upon completion of extraction, the SPME fibers were immediately inserted into the injector port for desorption and gas chromatography-flame ionization detector (GC-FID) analysis.

Gas chromatography analysis

The GC-FID system consisted of an Agilent 6890 Gas Chromatograph equipped with a CP-Volamine (30 m $\times$ 0.32 mm)

column. The injector and detector temperatures were set at 200 °C and 250 °C, respectively. Initially, the column temperature was maintained at 40 °C for 5 min, then increased by 10 °C/min to 120 °C and maintained for 2 min. Nitrogen, hydrogen, and air were used as the carrier gas at flow rates of 40, 40 and 400 mL/min, respectively.

Statistical analysis

All experiments were performed in triplicate ($n = 3$), and statistical analysis was conducted using IBM SPSS Statistics 23 (IBM, Armonk, NY, USA). All values were expressed as mean $\pm$ standard deviation (SD). A one-way analysis of variance (ANOVA) was conducted. Appropriate comparisons were made using the Student–Newman–Keuls test with significance defined as $p < 0.05$ for the ANOVA (Chun et al., 2014). Pearson's correlation coefficients were used to investigate the relationship between the change in the color of the freshness indicator and pH, VBN, TBARS, and TBC.

Results and discussion

Sensor response during storage

All color indicators were placed on the top of the beef sample package over a hole in the packaging in order to detect an increase in pH as volatile compounds (e.g. ammonia, dimethylamine, and trimethylamine) were released gradually into the package headspace, leading to a distinct color change from red to yellow. The sensors were monitored until no further discoloration was observed. Figure 2 shows the color changes of the freshness indicators during storage at 20 °C (we took a picture of sensors after 0 h, 12 h, 18 h, 24 h, 36 h, 48 h, 60 h, 72 h of storage) and the chromaticity values of each sensors are presented in Table 1. There were significant differences in the chromaticity values of the meat samples during storage, with the values of L , a , and b changing from 64.74 to 99.22, from 47.70 to -3.50 , and from -50.39 to -8.19 , respectively. In addition, ΔE gradually increased (as the originally red freshness indicators turned yellow) over the first 24 h of the experiment. Visual inspection failed to detect any difference in color between the sensors of different batch samples. We thus expect the proposed pH-

sensitive freshness indicator to be useful in improving the traceability system for freshness indicators and for Hazard Analysis Critical Control Points (HACCP; Yam et al., 2005).

pH, VBN, TBARS, and TBC analysis of the beef samples

In order to investigate the physicochemical properties of the beef samples, the pH, VBN levels, TBARS, and TBC of the samples at 20 °C were measured (Table 2). After death, muscle typically becomes more acidic (i.e. the pH decreases). The initial reduction in pH is caused by the accumulation of lactate, and the subsequent increase is caused by alkalization due to the release of the products of post-mortem proteolysis (Florek et al., 2007). As shown in Table 2, the pH of the beef samples significantly increased during storage ($p < 0.05$). At the beginning of the storage period, the initial pH was 5.52, which then decreased to 5.47, followed by an increase. Usually, after death, muscle becomes more acidic (pH decrease). Initial pH reduction is caused by lactate accumulation during the process and subsequent pH increase is due to the alkalization process due to the release of the primary product of proteolysis through post-translational changes. Similar results for the pH of beef samples have been reported by Kim et al. (2012b).

The initial pH decline is caused by lactic acid accumulation during glycolysis and the later pH increment is caused by progressing alkalization due to the release of basic products of protein breakdown throughout the post-mortem changes.

The pH increased with increasing storage time due to the putrefaction of the meat. Previous studies have reported that the pH is affected by the accumulation of VBN species originating from microbial activity (Castro et al., 2006). An increase in pH is thus associated with the rapid decay of meat during storage.

Because ammonia production due to the deamination of amino acids increases during spoilage, total VBN has frequently been used as an index for the decomposition of meat (Byun et al., 2003). The VBN levels for the beef samples during storage at 20 °C were determined using the Conway micro-diffusion technique from Kim and Shin (2011), and the results are summarized in Table 2. The VBN levels significantly increased during storage

Fig. 2 Changes in color of the freshness indicator during storage at 20 °C

Table 1 Chromaticity of the sensor in response to volatile basic nitrogen (VBN) according to spoilage of beef

	Storage time (h)					
	0	12	18	24	36	48
L	64.74 ± 5.29 ^{1),a,2)}	69.28 ± 5.10 ^{ab}	72.32 ± 2.80 ^b	87.63 ± 2.37 ^c	96.16 ± 1.33 ^d	97.66 ± 1.61 ^d
a	47.70 ± 6.57 ^a	43.72 ± 6.40 ^a	35.00 ± 2.20 ^b	13.86 ± 1.19 ^c	0.72 ± 2.41 ^d	0.25 ± 2.51 ^d
b	– 50.39 ± 8.77 ^a	– 44.57 ± 8.93 ^{ab}	– 38.48 ± 1.39 ^b	– 13.84 ± 1.72 ^c	0.33 ± 4.94 ^d	– 0.52 ± 3.04 ^d
△E	0.00 ^a	8.41 ± 0.08 ^{ab}	19.56 ± 10.78 ^b	54.83 ± 11.08 ^c	76.00 ± 9.34 ^d	77.47 ± 8.71 ^d

¹⁾All values are mean ± standard deviation of triplicate determinations²⁾Means with different letters in the same row are significantly different ($p < 0.05$) based on the Student–Newman–Keuls test

($p < 0.05$), beginning at 5.83 mg% and rising to 20.02 mg% after 24 h at 20 °C. Chun et al. (2013) also reported that VBN levels increase during storage. In Korea, the upper VBN limit for fresh meat is 20 mg% (Kim et al., 2012b). In the present study, the detection levels were accurate at the 24 h detection point.

Animal fats in meat are oxidized during storage and may affect the storage life. Rancidity has many causes, one of which is microbial activity (Singhal et al., 1997). TBARS can be measured in raw and cooked meat to indicate the extent of lipid peroxidation. Malonaldehyde (MDA) is a secondary decomposition product of several polyunsaturated fatty acids with three or more double bonds and reacts with TBA to form a stable pink coloration with a maximum absorbance at 532 nm (Sun et al., 2001). The TBARS levels of the beef samples presented in Table 2 show that there was a significant increase during storage ($p < 0.05$), from 0.71 mg MDA/kg at the start of storage to 1.48 mg MDA/kg after 24 h. It has previously been reported that a TBARS higher than 1.2 mg MDA/kg of TBARS represents the decay point (Kim et al., 2000).

The microbiological quality of meat is influenced by various factors, such as the animal's physiological state during slaughter, potential contamination during slaughter, temperature, and storage conditions (Sabow et al., 2016). Microbial analysis was performed based on TBCs (Table 2). It shows that, during the first 24 h, the TBC steadily increased from 3.81 to 7.06 log CFU/g at 24 h. According to Shin et al. (2006), spoilage takes place when the TBC reaches 6–7 log CFU/g and the level at which microbial spoilage occurs in beef is 9 log CFU/g.

Methylamine, dimethylamine, and trimethylamine analysis

Headspace solid phase microextraction (HS-SPME) with polydimethylsiloxane-divinylbenzene (PDMS-DVB) fibers was conducted, with fiber needles inserted into a GC-FID to determine methylamine (MA), dimethylamine (DMA), and trimethylamine (TMA) levels. SPME is a simple, efficient, and solvent-free method of analysis, and HS-SPME is a non-invasive form of SPME for the analysis of volatile analytes in gas mixtures. It has been shown that 65-μm PDMS-DVB fibers are suitable for determining VBN levels in SPME (Al-Masri and Al-Bachir, 2007).

For quantitative analysis, a series of volatile amines were used. The calibration curve is based on five different concentration levels (2.0, 5.0, 10.0, 20.0 and 40.0 μg/g), with varying amounts of internal standard added. Each point on the calibration curve is the average of three peak area measurements. The equations and squared correlation coefficients of determination (R^2), for which 0.99 represents good linearity, were as follows:

Table 2 Change in pH, volatile basic nitrogen (VBN), TBARS, and total bacterial count (TBC) analysis of beef during storage at 20 °C

	Storage time (h)							
	0	12	18	24	36	48	60	72
pH	5.52 ± 0.02 ^{1),a,2)}	5.49 ± 0.02 ^a	5.47 ± 0.03 ^a	5.57 ± 0.05 ^a	5.68 ± 0.07 ^a	5.69 ± 0.28 ^a	6.14 ± 0.07 ^b	6.38 ± 0.15 ^c
VBN (mg%)	5.83 ± 0.57 ^a	9.10 ± 0.81 ^a	13.07 ± 3.05 ^{ab}	20.03 ± 0.70 ^{bc}	26.31 ± 4.36 ^c	28.94 ± 0.89 ^c	43.31 ± 2.64 ^d	50.73 ± 1.07 ^d
TBARS (mg MDA/kg)	0.71 ± 0.03 ^a	0.84 ± 0.17 ^a	1.03 ± 0.75 ^{ab}	1.48 ± 0.01 ^{bc}	1.88 ± 0.01 ^c	2.63 ± 0.05 ^d	2.54 ± 0.02 ^d	2.46 ± 0.01 ^d
TBC (log CFU/g)	3.81 ± 0.34 ^a	5.19 ± 0.02 ^b	6.42 ± 0.06 ^c	7.36 ± 0.02 ^d	7.98 ± 0.12 ^e	8.33 ± 0.08 ^f	8.46 ± 0.10 ^g	8.74 ± 0.20 ^h

¹⁾All values are mean ± standard deviation of triplicate determinations²⁾Means with different letters in the same row are significantly different ($p < 0.05$) based on the Student–Newman–Keuls test **Fig. 3** Correlation between the trimethylamine concentration and ΔE value of sensor

$$\text{MA: } y = 0.0029x + 0.0061 \quad (R^2 = 0.9966)$$

$$\text{DMA: } y = 0.017x + 0.074 \quad (R^2 = 0.9924)$$

$$\text{TMA: } y = 0.0143x + 0.05 \quad (R^2 = 0.9940).$$

Unlike other food matrices (Chan et al., 2006), MA was detected after 60 h at 3.27 µg/g and increased to 4.21 µg/g after 72 h, while DMA was detected after 48 h at 0.25 µg/g and increased to 11.19 µg/g after 72 h. TMA illustrated a slightly different trend, being detected immediately at the start of the analysis (0 h = 0.44 µg/g) and increasing to 38.82 µg/g after 72 h. According to Shin et al. (2006), the concentration of TMA when meat was classed as off-flavored was 25 µg/g. There was also a strong correlation between ΔE and TMA concentration during beef storage (Fig. 3). Similar results for the TMA of meat samples have been reported by Xiao-wei et al. (2016).

Validation of the proposed method was conducted before using the standard solutions. The use of the five different concentrations (2.0, 5.0, 10.0, 20.0 and 40.0 µg/g) is indicated by a calibration curve. The R^2 of 0.99 indicates a higher linearity. The limit of detection (LOD) is defined as the lowest concentration with a signal-to-noise ratio of 3, while the limit of quantification (LOQ) is defined as the lowest concentration with a signal-to-noise ratio of 10 (Lee et al., 2015). In this research, the LOD was 0.16 µg mL⁻¹ for MA, 0.18 µg mL⁻¹ for DMA, and 0.12 µg mL⁻¹ for TMA, while the LOQ was 0.49 µg mL⁻¹ for MA, 0.53 µg mL⁻¹ for DMA, and 0.37 µg mL⁻¹ for TMA. The recovery measured using the peak area of stand was 99.51% for MA, 111.88% for DMA, and 115.07% for TMA.

Correlation of the color indicator with pH, VBN, TBARS, and TBC during storage

The correlation coefficients for the target indicators (pH, VBN, TBARS, and TBC) with the color of the sensors during storage at 20 °C are presented in Table 3. Strong correlations were observed between the color of the

Table 3 Correlation coefficient or coefficient of multiple correlations between pH, VBN, TBARS, TBC and color of the sensor

Parameter	L	a	b	ΔE
pH	0.715* $p = 0.046$	− 0.705 $p = 0.051$	0.589 $p = 0.125$	0.664 $p = 0.073$
VBN (mg %)	0.888** $p = 0.003$	− 0.882** $p = 0.004$	0.798* $p = 0.018$	0.854** $p = 0.007$
TBARS (mg MDA/kg)	0.956** $p = 0.000$	− 0.948** $p = 0.000$	0.922** $p = 0.001$	0.943** $p = 0.000$
TBC (Log CFU/g)	0.962** $p = 0.000$	− 0.965** $p = 0.000$	0.940** $p = 0.001$	0.960** $p = 0.000$

*Statistically significant at $p < 0.05$; **statistically significant at $p < 0.01$

freshness indicators and the parameters, with the strongest between TBC and L (0.962), a (− 0.965), b (0.940), and ΔE (0.960; all $p < 0.01$). The next strongest set of correlations was with TBARS (0.956, − 0.948, 0.922, and 0.943 for the L , a , b , and ΔE values, respectively; $p < 0.01$) and VBN level (0.888, − 0.882, 0.798, and 0.854 for the L , a , b , and ΔE values, respectively; $p < 0.01$ for all except b , which was < 0.05).

Even though the correlations between the changes in color and the indicators were good, they remained insufficient. The end point of the indicator has to correspond with the end point of product quality. A previous report by Shin et al. (2006) demonstrated that the microbial spoilage of beef begins when microbial levels reach 6.0–7.0 log CFU/g, and the level at which microbial spoilage occurs in beef is 9 log CFU/g. For this purpose, the microbiological shelf life of the beef samples was determined on the basis of the TBC, with 7 log CFU/g used as the limiting value for a microbiologically acceptable product. The sensory shelf life was defined using a VBN of 20 mg% as the upper limit, and the chemical shelf life was defined using TBARS and pH values of 1.2 mg MDA/kg and 6.2, respectively, as upper limits.

Using these criteria, the sensory, microbiological, and chemical shelf life was 24 h at 20 °C. The only exception was the chemical quality based on pH, which was still acceptable after 24 h of storage, but TBC, VBN, and TBARS had already exceeded the established limits. These results are similar to those reported by Byun et al. (2003), who observed a change in pH over the storage period, but its correlation with microbial counts was relatively low compared with that of other indicators such as VBN or TBA. The end-point of the freshness indicator corresponded to a TBC of 7 log CFU/g, a VBN level of 20 mg%, and TBARS of 1.2 mg MDA/kg.

A strong correlation between product quality and indicator color change was observed, meaning it was possible to develop a freshness indicator with end points matching

the microbiological, chemical, and sensory shelf-life of beef.

We conducted pH, VBN, TBARS, and microbial analysis to monitor the change in quality and the time of decay occurring during the storage of beef. During the storage period, the color of the freshness indicator changed from red to yellow after 24 h and content of VBN, TBARS, and TBC reached to 20.03, 1.48, and 7.36 mg% respectively. It was found that there was a strong correlation between the change in the color of the freshness indicator and content of chemical quality indicator in beef during storage. Therefore, it is possible to measure the freshness of meat products using the proposed freshness indicator. This study helps in the establishment of criteria for freshness indicators and in determining the suitability of various sensors in intelligent food packaging applications. Future research on this freshness indicator model should investigate its response to various environmental conditions, including damaged packaging, high temperatures, and dynamic temperature fluctuations.

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