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Development of a pH indicator composed of high moisture-absorbing materials for real-time monitoring of chicken breast freshness

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Abstract A colorimetric bromocresol purple dye-based pH-responsive indicator was developed to monitor the quality of chicken breast meat by direct surface contact. To prevent direct contact of the dye with the chicken breast and to improve its color change sensitivity, it was immobilized with polyvinyl alcohol and a high-absorbance material. The color of the pH indicator changes from yellow to blue and finally purple to indicate spoilage, which can be easily detected by the naked eye. The as-prepared pH indicator exhibited good response to pH changes on the surface of chicken breast meat, and no migration of the dye from the indicator onto the surface of the chicken was observed. This pH indicator exhibits excellent feasibility for real-time, direct-contact monitoring of the freshness and quality of various foods.

Keywords: chicken breast, color change, freshness indicator, quality change

Introduction

Consumer demand for fresh and safe food products is continuously increasing. As the number of food products increases and their packaging types diversify, the food industry must address concerns regarding the shelf-life and safety of packed food products (1-5). Consequently, innovative and creative packaging technologies that exhibit active and intelligent functions, both in terms of shelf-life extension and quality monitoring, have been developed (1,5). Recently, considerable research efforts have focused on the development of various measures to ensure food safety, improve quality, and warn manufacturers and consumers of potential problems during food transport, storage, and usage. For instance, the integrity of food packaging and time-temperature indicators (TTIs) are invaluable for monitoring the quality and safety of packaged foods during transport and storage, and several such technologies are already commercially available (1,5,6). Thus, smart packaging containing intelligent TTIs that provide information on the thermal history of packaged food products during storage and transportation are invaluable to the food industry, retailers, and consumers.

Retail and consumer level spoilages and losses in the meat industry mainly occur owing to unsuitable packaging, food wastage, and improper storage, the latter is often caused by lack of consumer awareness regarding correct storage conditions. Consumers often decide meat quality and assess safety for consumption based on three sensory attributes: appearance, texture, and flavor (7). The visual appearance of a meat product has the greatest influence on consumer acceptance, and any objectionable change in the surface color of meat may result in product rejection. However, sensory evaluation is subjective and expensive in most cases. Microbial methods for detecting spoilage microorganisms and chemical methods that measure chemical changes, such as evolved volatiles and pH change, are not suitable for measuring deterioration upon early harvesting and slaughtering. Therefore, rapid and simple methods for the measurement of meat freshness are required (8). Since meat spoils quickly during storage and transport at ambient temperature, a packaging technology containing a real-time indicator for identifying spoilage would be particularly useful in ensuring the quality and safety of packaged food products (1,4,5,9).

Among the various meats, chicken has shown increased consumption in many countries owing to its association with a more healthy diet, the fact that it is a relatively inexpensive protein source, and because it may be presented in many convenient and appealing forms in the modern western diet (8,10). Chicken is a highly perishable food and is usually spoiled within a week of slaughtering, regardless of storage conditions. This spoilage is mainly due to microorganisms and is strongly dependent on the initial quality of the meat (9,11). Therefore, reliable and simple methods for assessing the microbiological properties and/or real-time freshness of chicken, and thus indicating safety for consumption, would benefit manufacturers, retailers, and

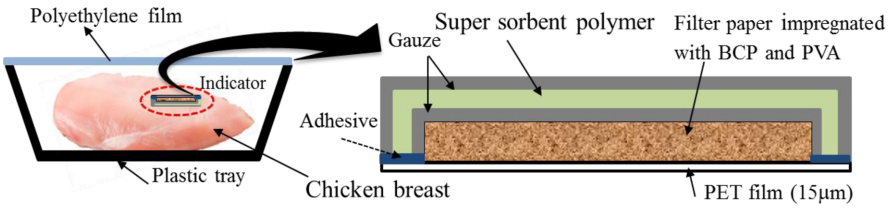

Fig. 1. Structure of the pH indicator prepared in this study

consumers.

Shelf-life studies of highly perishable meat products are usually performed by evaluating the microbiological and sensory qualities of the product as a function of storage conditions such as time, temperature, and air composition. Because these microbiological analyses are expensive and time consuming, various methods involving chemical changes caused by microbial growth during storage have also been investigated as meat quality indicators (9,10). For example, the ripening and/or spoilage of meat, which cause a decrease in electrolyte dissociation, an increase in the concentration of buffering proteins, and the formation of ammonia, may be observed in the form of an increase in pH (12).

Color-based pH indicators have potential for use as indicators of microbial growth and changes in metabolite levels originating from food spoilage and protein degradation for freshness monitoring (2,8). This method can be used for on-package monitoring of food spoilage. For instance, indicators with pH sensitive dyes immobilized onto plastic films or cellulose membranes have been proposed as fish-spoilage indicators (13,14). This methodology works by detecting gradual changes in pH caused by the spoilage of fish, as volatile basic amines evolved into the food package headspace cause an increase in the pH and subsequently the color of the indicator changes from yellow to blue, which is easily detected by the naked eye. Studies using the same principle have been reported by Kuswandi *et al.* (2).

However, to the best of our knowledge, research on real-time monitoring of product freshness has mainly focused on the color changes of indicators in packaging headspace (2-4,14-16). On-package sensors containing pH-sensitive dyes only respond to volatile spoilage compounds in the headspace evolved from the surface of foods. In other words, these non-direct-contact methods require some headspace and respond only to volatile spoilage compounds. Therefore, these technologies are limited as they do not respond to compounds dissolved on the surface of products. However, for safety reasons, the problem of dye migration into food products must be taken into account for direct-contact-type indicators.

The purpose of this study was to develop a real-time pH-indicating monitor based on bromocresol purple (BCP) suitable for direct contact with chicken breast surfaces. To prevent the migration of BCP into the chicken breast, it was immobilized with polyvinyl alcohol (PVA) and a high-absorbance pad. As shown in Fig. 1, the pH indicator in this study was designed as a three-layered structure with a super-absorbent pad, which was used to prevent direct contact of the dye with the chicken breast and to improve the color change sensitivity of BCP. The effectiveness of the pH indicator was examined by attaching it to the surface of chicken breasts stored at 4 and 10°C. The indicator response was correlated with pH, volatile basic nitrogen (VBN) content, bacterial growth, and changes in the surface color of the chicken breast. This color-based pH indicator was successfully prepared and tested for the visual monitoring of the real-time freshness of chicken breast in direct contact mode.

Materials and Methods

Materials BCP (dye content 90%) as the dye, 98.0–99.5% hydrolyzed PVA (average molecular weight: 17,500 g/mol) as the binder, and filter paper (Whatman™ Filter Paper No. 42; Whatman plc, Maidstone, UK) as the indicator carrier were purchased from Sigma-Aldrich Co. (St. Louis, MI, USA), OCI Co., Ltd. (Incheon, Korea), and GE Healthcare UK Ltd. (Little Chalfont, UK), respectively. Deionized water was used as the solvent for PVA. A 15 μm polyethylene terephthalate (PET) film was supplied by Soojoung Co., Ltd. (Gwangju, Korea). A high-absorbance pad with a three-layered structure of gauze/super-sorbent polymer (polyacrylamide)/gauze was purchased from Yuhan Kimberly Co., Ltd. (Daejeon, Korea).

Fabrication of pH indicator First, BCP was immobilized on a filter paper using the absorption method (9) by simply immersing the filter paper in 20 mL BCP/PVA/deionized water solution (0.03 g/10 g/90 mL) for 1 h at ambient temperature. Then, the BCP/PVA/filter paper was dried in an oven at 70°C for 24 h. The filter paper impregnated with BCP and PVA was then cut to the desired shape and size and placed on the PET film. It was covered with the pad, and the PET film and pad was tightly sealed at the edge with adhesive tape. As shown in Fig. 1, the dimensions of the obtained indicators were approximately 10 mmx10 mm and yellow in color.

Physicochemical and microbiological changes Fresh and boneless chicken breast of normal pH (5.9–6.0) was purchased from a local poultry meat shop (Wonju, Korea). The chicken breast was cut into 100 g portions, placed on plastic trays, and wrapped in low density polyethylene film that had been sterilized in the clean bench under UV-B conditions for 1 h, as shown in Fig. 1. To investigate the color

change in the pH indicator due to quality changes during storage, the as-prepared indicator was placed directly on the surface of the chicken breast. The samples were stored under two different temperature conditions: 4±0.5 and 10±0.5°C. To correlate the color change of the indicator with the deterioration in the quality of the chicken breast during storage, the VBN content, change in pH, total plate count, *Pseudomonas* spp. microbial growth, and changes in the surface color of the chicken breast were monitored as important physicochemical and microbiological parameters (2,8,17-19).

The VBN content, expressed as mg VBN 100 g^{-1} sample, was measured using the Conway micropipette diffusion method (20). The measurements were performed in triplicate. Determination of the pH directly on the surface of the chicken breast was conducted using a portable pH meter (PT-15; Sartorius AG, Goettingen, Germany) fitted with a combination insertion electrode.

For microbiological analysis, the total plate count and *Pseudomonas spp.* microbial growth of the chicken breast samples were investigated. On each sampling day, 10 g sample was aseptically transferred to a 100 mL sterile bag, 90 mL sterile 0.1% (w/v) bacteriological peptone water was added, and the mixture was homogenized in a stomacher for 3 min at low speed and room temperature. Serial decimal dilutions using 0.1% peptone water were prepared, and duplicate 1.0 mL samples at appropriate dilution ratios were poured on all-purpose and selective agar plates. Coliform count plate Petrifilm (3M, Elyria, OH, USA) and Pseudomonas agar base (Oxford, MS, USA) were used for total aerobic bacteria and *Pseudomonas spp.*, respectively. The incubations for total aerobic bacteria and *Pseudomonas spp.* were performed at 37°C for 48 h and at 20°C for 96 h, respectively, under aerobic conditions. Microbial count determination was performed in triplicate.

The surface color of the chicken breast samples during storage was measured using a chromatic meter (TES-135; TES Electric Electronic Co., Ltd., Taipei, Taiwan) and is reported as Hunter *L** (lightness), *a** (redness), and *b** (yellowness) values as a function of storage time. Each sample determination was repeated three times.

Results and Discussion

pH measurement The pH values for the surface of the chicken breast samples during storage at 4 and 10°C were determined using a pH meter and are shown in Fig. 2. At the beginning of the storage period, a relatively low and constant pH (pH=5.9–6.0) is obtained, representing the fresh state of the chicken breast samples. At the lower storage temperature of 4°C, the pH increases to 6.1 and remains almost constant for 6 days, after which it sharply increases with each storage day. Moreover, the pH of the samples stored at 10°C increases linearly and steadily during storage, and the 3-day value is almost the same as the 6-day value of the sample stored at 4°C. Depending on the storage temperature, the change in pH value may be related to the apparent change in the quality of the chicken breast, originating from physicochemical and microbiological deterioration (8,9,17,19).

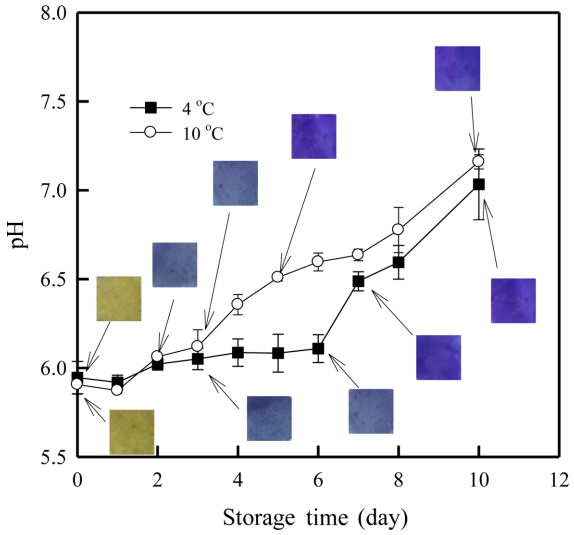

Fig. 2. pH changes in the chicken breast surface and their relationships with the color changes of the pH indicator with storage time

A previous study reported that a pH of approximately 6.3–6.5 indicates the onset of chicken breast deterioration and suggested that an increasing pH value is related to accumulation of trimethylamine and other VBN species originating from the decarboxylation of specific amino acids by microbial activity (17,21). An abrupt increase in pH is associated with rapid spoilage of chicken breast and an increase in mean pH value over the storage period. pH changes are also closely correlated with the total viable count.

VBN measurement During the storage of chicken meat, volatile amines including VBN species or ammonia are formed mainly because of biochemical changes originating from protein breakdown by proteolytic enzymes (8,21). As shown in Fig. 3, the VBN content of the chicken breast stored at 4°C remains almost constant until day 6, after which it increases rapidly with storage time. In contrast, the VBN content of the samples stored at 10°C shows a linear proportional relationship and strong dependence on the storage time. As shown in Fig. 2, these changes are consistent with those in the pH values. For samples stored at 4°C, the VBN values are 4 mg% on day 6, 23 mg% on day 7, and 60 mg% on day 10. The VBN values of the samples stored at 10°C are 4 mg% on day 3 and 30 mg% on day 4. This indicates that the biochemical properties of the chicken breast in this study deteriorate considerably after 6 days at 4°C and 3 days at 10°C. As the threshold limit value for chicken breast is 20 mg/100 g after 6 and 3 days at 4 and 10°C, respectively, the chicken breast samples are no longer appropriate for human consumption under the conditions of the experiment. This is comparable to the results obtained in the studies by Nopwinyuwong *et al.* (15) and Rukchon *et al.* (19). The dramatic change in VBN content could be related to the increased growth of microorganisms, which is accompanied by a

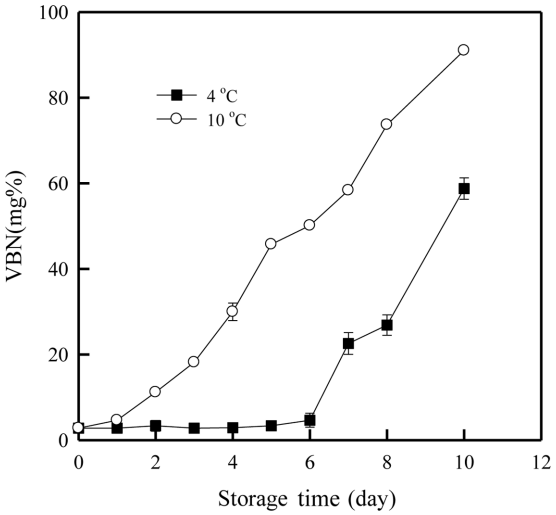

Fig. 3. Changes in the VBN content of chicken breast samples stored at 4 and 10°C

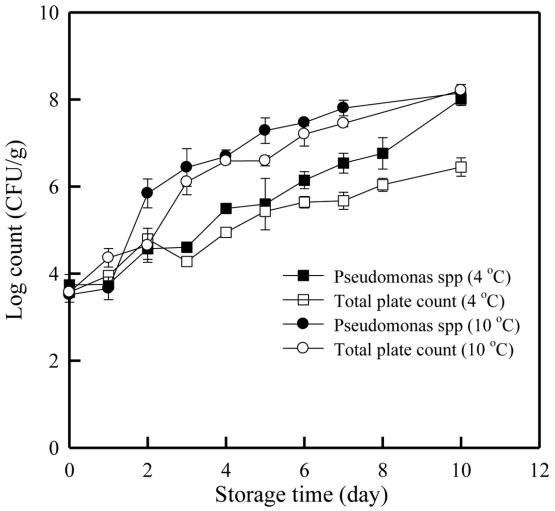

Fig. 4. Changes in total plate count and *Pseudomonas spp.* levels of chicken breast stored at 4 and 10°C

large change in chicken breast quality. The sudden increase in microorganisms might be attributed to spoilage changes, as confirmed by the increased VBN levels. The increase in VBN levels causes an increase in pH in the chicken breast and packaging. From these results, it can be speculated that freshness changes in chicken breast can be monitored by introducing the as-prepared pH indicators.

Microbial growth At the beginning of the storage time, specific spoilage microorganisms exist in low quantities and occupy only a minor part of the natural microflora. As storage time increases, these generally grow faster and generate various metabolites responsible for off-odors, off-flavors, and color and texture changes, finally resulting in sensory rejection (19). The total plate count and *Pseudomonas spp.* level, which were expected to be the specific spoilage microorganisms, were investigated as the most important microbial parameters of chicken breast. As shown in Fig. 4, both the total plate count and *Pseudomonas spp.* level increase continuously with storage time. As expected, the increase is more prominent at the higher storage temperature. Specifically, *Pseudomonas spp.* can grow continuously under these storage conditions. The limit of acceptability for human consumption as the onset index of food spoilage is 6.0–7.0 log CFU/g microorganisms for fresh poultry (19,22).

Considering the VBN content and food spoilage by microorganisms, the shelf-life of the chicken breast is approximately 6 and 3 days for the samples stored at 4 and 10°C, respectively. Our results are comparable with those proposed by Rukchon *et al*. (19), i.e., the shelf-life of skinless chicken breast is 6.12 and 2.78 days at 4 and 10°C, respectively.

Surface color of chicken breast Depending on the storage conditions, the color changes of products are less critical but still important as a parameter that indicates product uniformity and informs consumer acceptance (21,23). The muscle color of meats is related to structural changes in myoglobin molecules (7) and is affected by several factors, including feeding level, diet composition, age, sex, physical activity of the animal, antioxidant accumulation, glycogen storage, genetic variability, pre-harvest environment, pre-slaughter handling and stunning method, post-harvest spraying, and packaging techniques. Chemical changes in meat, such as protein denaturation, oxidation, hydrolysis, changes in pH, and enzyme action, are also significant factors affecting its color. The parameters L^*, a^*, and b^* indicate lightness, red (+) or green (–) coordinate, and yellow (+) or blue (–) coordinate, respectively (24). As shown in Fig. 5, lightness values slightly increase during the first 3 days of storage and then decrease linearly below the initial value. Samples stored at 10°C show the same trend as those stored at 4°C but with a smaller change range. The Hunter a^* values at 4 and 10°C increase significantly during days 2–3 and then level off and increase with storage time. Moreover, The Hunter b^* values at 4°C decrease slightly and level off until day 6, after which they decrease with storage time. Those at 10°C decrease during the first 3–4 days rapidly until they reach those of day 10 at 4°C, and then remain constant. These color indices indicate that the surface color of the chicken breasts changes from pink to red violet and finally dark purple or bluish-red, i.e., their lightness decreases with storage time. Color changes in chicken breast are related to quality changes and are highly dependent upon the storage day and temperature. The chicken breast stored at 4°C shows relatively small changes in lightness (ΔL^*) and greater changes in yellowness (Δb^*), which indicates that less change in quality occurs at low temperature, as expected. However, the b^* values show an apparent drop during days 6–8 and 1–4 for the samples stored at 4 and 10°C, respectively, which is related to the proliferation of *Pseudomonas spp.*, as shown in Fig. 4.

Color change of the pH indicator In Fig. 6, the changes in Hunter

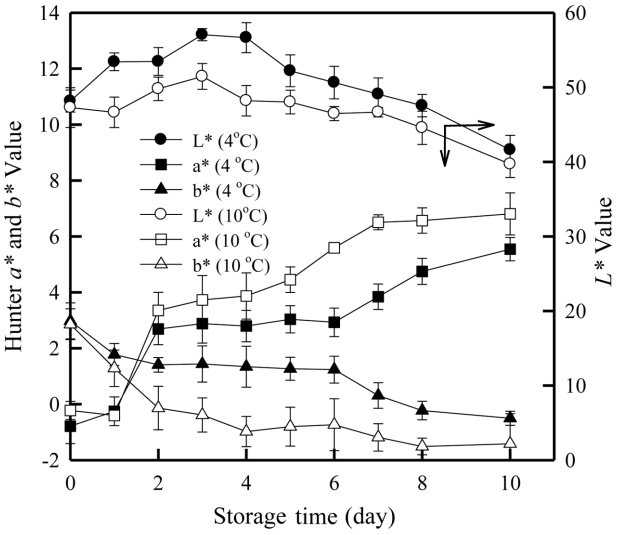

Fig. 5. Changes in the surface color of chicken breasts during storage at 4 and 10°C

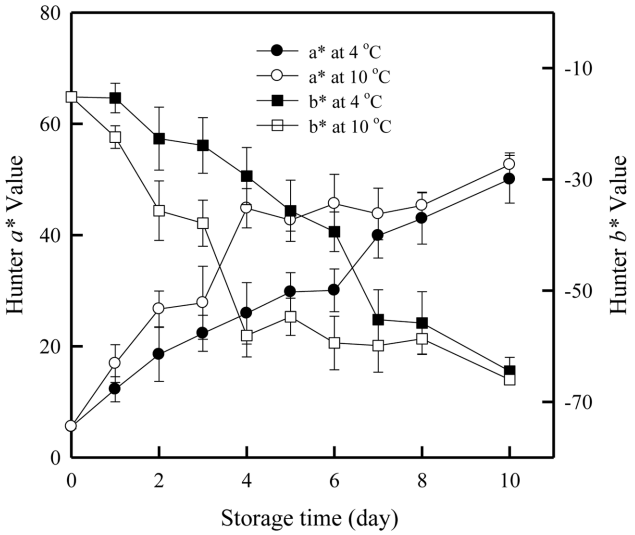

Fig. 6. Changes in Hunter color values *a** and *b** of indicators directly attached to the surface of chicken breasts during storage at 4 and 10°C

color *a** and *b** of the as-prepared indicators directly attached to the surface of the chicken breast at 4 and 10°C are depicted as a function of storage time. The labile proton of the dye is dependent on pH, and the dye is easily deprotonated in a basic environment. This causes a shift in the wavelength maximum of the dye and a concurrent color change (13). The Hunter *a** values of samples stored at both temperatures increase with time, whereas the Hunter *b** values decrease. This indicates that the redness of the indicator increases and the yellowness decreases with time; therefore, the color of the indicator changes from yellow to blue and finally purple. The color change of the indicator with storage time at 10°C shows the same pattern as that at 4°C, although the rate of color change is higher at increased temperature. This relates to color changes of the indicator as a result of quality changes of the chicken breast, which are strongly dependent on the storage temperature. As shown in Fig. 6, the increase in Hunter *a** value and decrease in Hunter *b** value for the indicator at higher temperature storage conditions are clearly apparent at a shorter storage time.

Changes in the freshness indicator color in response to changes in pH due to spoilage of the chicken breast are shown in Fig. 2. The pH indicator is yellow at pH 6.0 and changes to blue at pH 6.2 and to dark blue or purple at pH 6.6. As expected, the as-prepared pH indicator shows good response to pH change on the surface of the chicken breast and accumulation of VBN species during storage. Furthermore, there are no visual differences in color changes between the samples stored at 4 and 10°C, i.e., the visual color of the pH indicator changes only according to pH on the surface of the chicken breast. No migration of the dye from the indicator onto the surface of the chicken breast is detected after 10 days of storage. This indicates that the BCP dye is successfully entrapped within the high-absorbance materials and filter paper in our system, and that the as-prepared pH indicators do not show migration problems in our product-indicator direct-contact system.

The pH-responsive color change of the as-prepared indicator shows excellent response to pH change on the surface of the chicken breast and the VBN contents, which are strongly correlated to the proliferation of spoilage microorganisms. Thus, the quick and direct detection of chicken breast spoilage by the pH indicator attached to its surface is highly feasible using the pH responsive freshness indicator developed in this study. Furthermore, this prototype freshness indicator shows direct color changes to indicate the degree of freshness of the chicken breast and is therefore appropriate for predicting the shelf-life of products. After the 10-day storage test, no migration of the dye from the indicator to the chicken breast surface is detected, indicating that the BCP dye is well entrapped in the pH indicator system. Thus, our newly developed pH indicator containing high-moisture-absorbing materials shows excellent feasibility for real-time monitoring of the freshness of various foods, including chicken breast.

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Disclosure The authors declare no conflict of interest.

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