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Development of a pH-Sensitive Indicator Label for the Real-Time Monitoring of Chicken Meat Freshness

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ABSTRACT

Introduction: Intelligent packaging could be used to control the environment of food products and inform consumers of food conditions. The present study aimed to design a simple pH-sensitive label in chicken package to demonstrate food spoilage by color change.

Bromocresol green (BCG) is a pH-sensitive color indicator, which was used to detect chicken spoilage and attached to the inner side of the package. Chemical (pH and total volatile base nitrogen [TVBN]), microbial, and sensory analyses were performed at the intervals of zero, three, five, seven, eight, and 10 days of storage at the temperature of 4°C.

Methods: On the first day, the TVB-N was 19 mg/100g, while it was measured to be 29 mg/100 g on day eight and 46 mg/100g on the last daIntelligent packaging could be used to control the environment of food products and inform consumers of food conditions. The present study aimed to design a simple pH-sensitive label in chicken package to demonstrate food spoilage by color change.

Results: Bromocresol green (BCG) is a pH-sensitive color indicator, which was used to detect chicken spoilage and attached to the inner side of the package. Chemical (pH and total volatile base nitrogen [TVBN]), microbial, and sensory analyses were performed at the intervals of zero, three, five, seven, eight, and 10 days of storage at the temperature of 4°C. On the first day, the TVB-N was 19 mg/100g, while it was measured to be 29 mg/100 g on day eight and 46 mg/100g on the last dayy. According to the obtained results, the changes in the BCG color due to chicken spoilage were detectable by the naked eye, and the bacterial count was estimated at 6.62 Log10CFU/g. On day 10, the color of the indicator turned blue, and the total bacterial count reached approximately7.20 Log10CFU/g. In addition, significant correlations were observed between the TVBN, pH, and microbial and sensory analyses in the studied samples with the changes in the color of the indicator during storage.

Conclusion: Due to the increased TVBN during storage, the color of the BCG indicator changed from yellow to blue. Therefore, the BCG indicator could be employed as an inexpensive, simple label to show the freshness of chicken meat.

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Introduction

Microbial contamination of food products may decrease their shelf life and increase the risk of food borne diseases (1).Chicken meat contains low levels of saturated fats and is considered to be highly perishable, deteriorating within 4-7 days of storage at refrigerated temperatures (2). The microorganisms involved in chicken meat spoilage include various bacteria (e.g., *Pseudomonas* spp. and *Shewanella putrefaciens*), aerobic bacteria, and yeasts. During the storage of chicken meat, proteolytic and lipolytic enzymatic activities of microorganisms could also cause the spoilage and decomposition of the proteins and fats.

The traditional methods used to determine food quality are often time-consuming(e.g., physical, chemical, and microbiological methods) (3), chemical indicators have been recommended as quality assessment tools. Some chemical indicators are used to evaluate the quality of meat; such example is biogenic amines, which are

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the products of the degradation of nucleotides and volatile acids. These compounds may serve as quality indicators for fresh chicken meat during storage (2, 4). Furthermore, various products of bacterial degradation are known as total volatile base nitrogen (TVBN), such as trimethylamine (TMA), dimethylamine (DMA), and ammonia (5).

Common packaging often protects food products against destructive environmental factors, such as heat, moisture, microorganisms, and dust (6-8). Intelligent/smart packaging is a system of packaging that could monitor the environment of food products, thereby providing data on the quality of food during storage, transportation, and distribution(1, 9). Therefore, changes in the pH of food packaging cause the color of these indicators to change, indicating food spoilage (10).

Today, food packaging indicators are essential for consumers to be informed of the freshness of food products. When chicken meat loses its freshness in an alkaline environment, volatile amines are emitted from the meat, changing its pH. In intelligent packaging, pH-sensitive dyes (e.g., bromothymol blue, methyl red, bromothymol blue, phenol red, and bromocresol) change color with increased pH due to the production of TVBN during food spoilage; this is detectable by the naked eye (11, 12).

Bromocresol green (BCG) is a pH-sensitive dye, which could demonstrate the TVBN content through color change from yellow (acidic environments) to blue(alkaline environment)(10, 13).BCG has been used as a pH indicator within the range of 1.5-8.5(14).The wide ranges of pH detection by BCG (most obvious sequential color change)render it an appropriate pH indicator(15).Moreover, colorimetric indicators could be applied for the detection of chicken meat freshness as rapid, cost-effective, reliable, and non-destructive methods.

The present study aimed to design an inexpensive, simple label for chicken meat packaging the color of which changes over time to signal spoilage.

Materials and Methods

Sample Preparation

Fresh chicken meat was purchased from a market and immediately transferred to the laboratory in an ice box. The packages with the indicator were sterilized by the UV beam, and100±5 grams of the samples were placed in sterile food containers with the indicator attached to the inner side of the lid with no connection to the meat. Afterwards, the samples were stored at the temperature of 4°C, and the analyses were conducted at the intervals of zero, three, five, seven, eight, and 10 days.

Indicator Design

At this stage, two milliliters of the BCG indicator (0.1%) was mixed with 40 milliliters of distilled water and 0.1 gram of edible gelatin (0.25%), and the mixture was placed on a transparent polypropylene polymer layer. Gelatin was an edible and harmless material with adhesion properties, which was used to immobilize the BCG.

Chemical Analysis

The pH meter was calibrated at points four, seven, and 10. Following that, five grams of the sample was mixed with 45 milliliters of distilled water using a stomacher and filtered through a filter paper. In each measurement, the electrodes were completely immersed in the homogenized chicken meat samples (16).

TVBN was performed using the steam distillation method as described by Mirshekari with some modifications. To do so, 10 grams of the homogenized samples was distilled in the presence of MgO, and the distillate containing the aqueous solution of boric acid and the indicator (methyl red and methylene blue) was titrated using sulfuric acid until the pink color appeared. TVBN was determined by multiplying the amount of the consumed acid by 14 (17), and the correlations between the indicator color change and chemical test results were evaluated.

Microbial Analysis

At this stage, 25 grams of the chicken meat samples was divided into smaller pieces in sterile conditions and homogenized in 225 milliliters of sterilized peptone using a stomacher for three minutes. Afterwards, the serial dilution was prepared, and the total viable counts were determined using the plate count agar (PCA). Following that, the samples were incubated at the temperature of 37°C for 48 hours (18).

Sensory Analysis

Sensory qualities were evaluated by five trained panelists, including the odor, color, appearance, texture, and overall acceptance of the meat samples. The assessment of the sensory properties of the samples was carried out immediately after opening the packaging. The panelists were asked to grade the chicken meat samples based on a five-point scale, with scores 4-5 indicating favorable quality, score three indicating moderate quality with some defects, score two interpreted as poor quality, and score one indicating dangerous for consumption (19).

Indicator Color Number

In order to determine the color number at different intervals, the image of the indicator was captured at the distance of 10 centimeters using a 13-megapixel digital camera. Afterwards, the sensor color was read in the Photoshop software(20).

Statistical Analysis

Data analysis was performed in SPSS version 20 and Excel software. The correlations between the variables were evaluated using repeated measures ANOVA and Spearman's correlation-coefficient.

Results

Chemical Changes

Positive correlations were observed between the pH changes, color indications, and chicken meat spoilage in all the available samples with the shelf life of the products. On the first day of storage, the pH of the chicken meat samples was 5.8, and the indicator color was yellow. After increasing the storage time, the pH slowly increased as well (Figure 1). On the third day, the pH reached 6.03 and increased slightly on day five until reaching 6.7, which was higher than the permissible limit on day eight, with the indicator color turning green. On the last day, the pH reached seven, and the chicken meat samples were completely spoiled; it is also notable that since day nine, the indicator color had turned blue.

According to the obtained results, TVBN was within the permissible range on the first day (19 mg N/100 g of meat; indicator color yellow), while it increased over time (Figure 2). On day eight, TVBN was measured to be higher than the allowable limit (29 mg N/100 g of meat; indicator color green), while it continued to increase to 46 mg N/100 g of meat on the last day. Since day nine, the indicator color turned blue, and the increased TVBN during chicken meat spoilage caused the indicator color to change. According to the information in Table 1, the TVBN changes were positively correlated with the storage time and indicator color in all the studied samples ($P<0.05$).

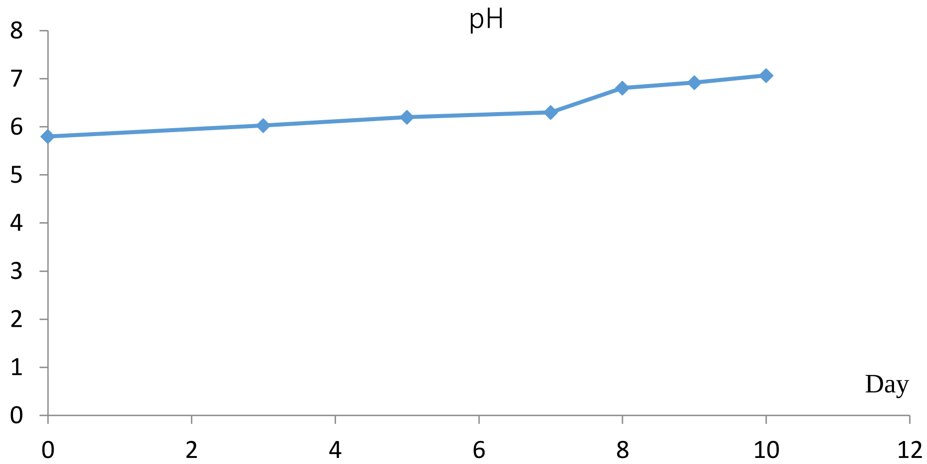

Figure1.pH of Chicken Meat Samples during 10 Days of Storage at Refrigerated Temperature (4°C)

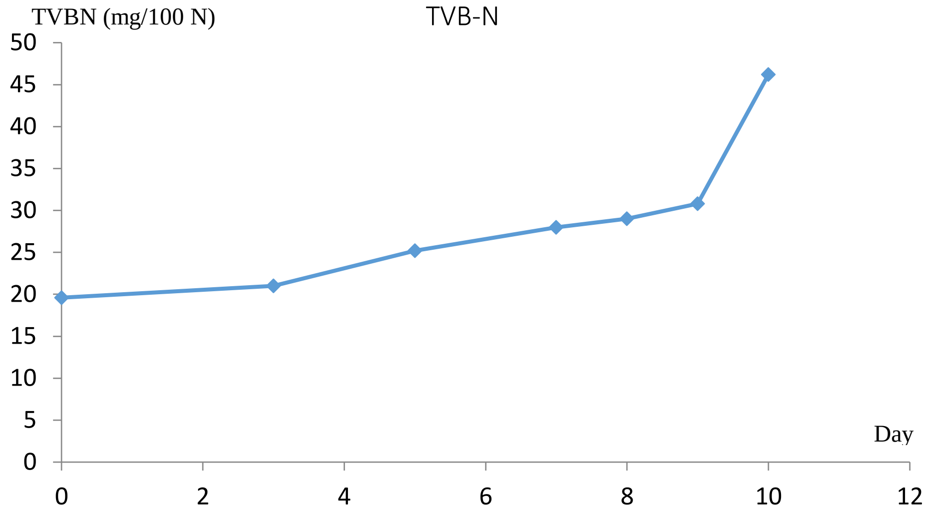

Figure2.TVBN in Chicken Meat Samples during 10 Days of Storage at Refrigerated Temperature (4°C)

Microbial Changes

Positive correlations were observed between microbial changes, chicken meat spoilage, and indicator color in all the samples (Figure 3). At the outset, the total bacterial count was estimated at 3.90 Log_{10}CFU/g (indicator color yellow), which slowly grew with the increased storage time of the chicken meat. On day eight, the green color appeared, and the bacterial count was observed to be6.62 Log_{10}CFU/g. On the last day, the indicator color turned blue, and the total count reached approximately 7.20 Log_{10}CFU/g.

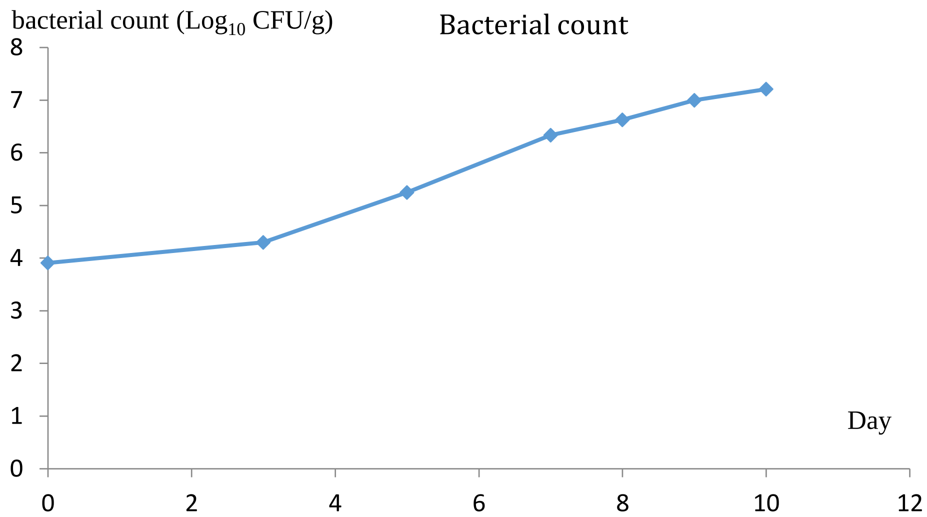

Figure 3. Total Bacterial Count in Chicken Meat Samples during 10 Days of Storage at Refrigerated Temperature (4°C)

Sensory Changes

A significant, positive correlation was observed between the sensory analysis (odor, color, appearance, texture, and total acceptance of the meat samples) with the storage time, chicken meat spoilage, and indicator color in all the available samples (P<0.05).According to the results of the present study, sensory analysis changed from highly favorable to extremely poor, and the indicator color started changing color, turning blue(Figure 4).

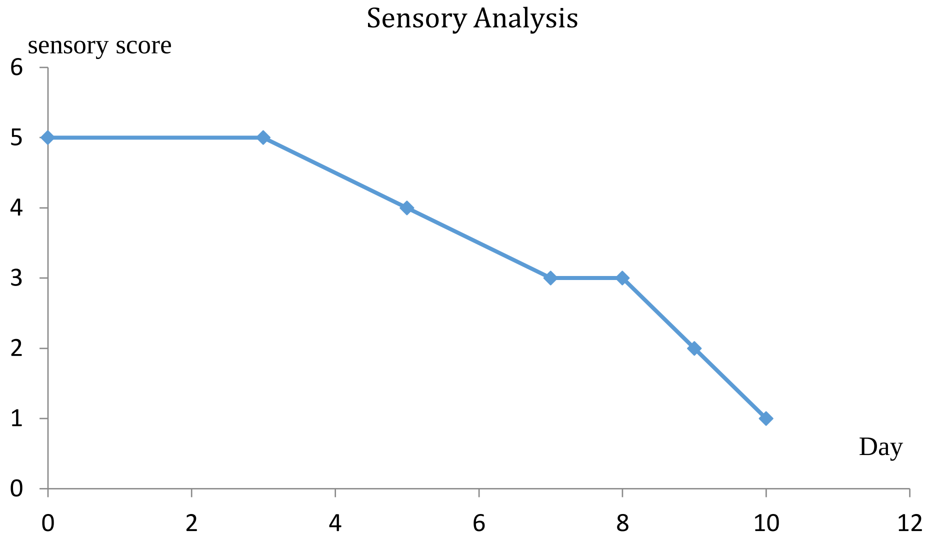

Figure4. Sensory Analysis in Chicken Meat Samples during 10 Days of Storage at Refrigerated Temperature (4°C)

Indicator Color Changes during Storage Time

In the current research, increased storage time at the temperature of 4°C caused no color change on days one and seven, and the yellow color remained constant. From day eight onwards, the indicator color turned green due to the increased microbial load, pH, and TVBN. Since day nine, the color became blue, and the color of the indicator remained permanent with no changes (figures 5& 6; Table 1).

Figure 5. Color Changes of pH Indicator and Edible Gelatin in Healthy (yellow), Spoiling (green), and Spoiled Chicken Meat Samples (blue) (right to left)

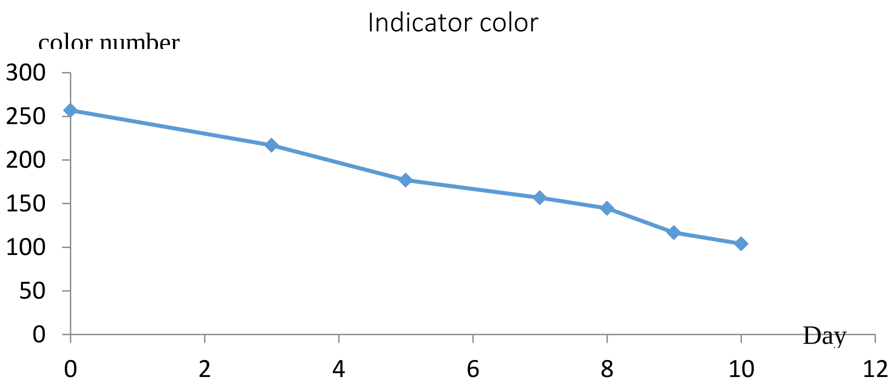

Figure6. Color Number of Indicator in Chicken Meat Samples during 10 Days of Storage at Refrigerated Temperature (4°C)

Table 1. Correlation-coefficient between Color Number of Indicator, TVBN, pH, Microbiological Log Count, and Sensory Analysis

	TVBN	pH	Bacterial Count	Sensory Analysis	Indicator Color
TVBN	-				
pH	0.932*	-			
Bacterial Count	0.982*	0.945*	-		
Sensory Analysis	0.917*	0.954*	0.974*	-	
Indicator Color	0.917*	-0.941*	-0.979*	-0.964*	-

Discussion

In the past decades, use of smart packaging and freshness indicators to monitor food quality has been widely investigated (10, 20). Although various pH-sensitive dyes have been applied in some studies, the response of freshness indicator depends on different physicochemical parameters, such as the dye type, color binder, and additives(20).In the present study, a simple combination of BCG and gelatin was incorporated into chicken meat packaging. Due to the spoilage of chicken meat during refrigerated storage, TVBN reached 29 mg/100g (standard of chicken meat: 28 mg N/100 g). Therefore, higher TVBN was associated with the decreased freshness of chicken meat.

The findings of the current research were indicative of a significant association between the microbial load and volatile nitrogen compounds in the fresh chicken meat. Due to spoilage, microorganisms produce hydrogen sulfide, dimethyl disulfide, ammonia, biogenic amines (e.g., putrescine and cadaverine), trimethylamine, and dimethylamine, which increase TVBN. Therefore, increased storage time and spoilage of chicken meat were associated with higher TVBN, and the measurement of TVBN indicated the freshness of the chicken meat samples during refrigerated storage (2, 21).

According to the results of the present study, changes in the total bacterial count, pH, and TVBN caused the color change of BCG, which is consistent with the findings of Kuswandi et al. (1) and Morsy et al. in fish samples(22).In the mentioned studies, the colorimetric indicators reacted to the compounds of the microbial metabolites of spoiled fish, thereby leading to their color change (1, 22).

In another research, Kuswandi et al. reported that changes in the methyl red color were associated with the high microbial load of broiler chicken meat, and the indicator could clearly demonstrate the spoilage of chicken meat inside the package with the color changing from red to yellow(23).In the mentioned study, Kuswandi used a label for fish spoilage based on the polyaniline film, indicated that higher microbial load in the fish sample tissues and production of a volatile base were associated with the color change of the film (1).In the current research, the indicator color changed from yellow to blue due to chicken spoilage and microbial growth. In this regard, Lee et al monitored chicken breast spoilage using the Tyvek sheet and RGB dye, reporting that the TVBN of the chicken breast stored at the temperature of 4°C remained unchanged for four days, reaching 20 mg/100g on day seven (20).

In the present study, TVBN was estimated at 19 mg/100g on the first day, reaching 29 mg/100 g on day eight and 46 mg/100g on the last day of storage. According to the obtained results, the changes in the BCG color due to chicken spoilage were detectable by the naked eye (yellow to blue).In another study; Soni et al. investigated the BCG purple indicator dye to monitor the chicken meat freshness during storage at the temperature of 4°C. The indicator color changed from light yellow to purple on day seven of storage due to the generation of basic gases inside the packages. Additionally, pH and TVBN reached 6.34 and 20.91 mg/100g, respectively after seven days (24); these findings are consistent with the results obtained by Kim et al. (25).

In the previous studies in this regard, pH-sensitive dyes have been loaded into various layers to show freshness, while in the current research, a simple combination of dye and gelatin was applied. In the study by Shukla et al., the color changes of bromophenol blue for the monitoring of buffalo meat were investigated, and the color response of the indicator was associated with the concentration of accumulated TVBN in the head space of the packaging; this is in line with the results of the present study. Furthermore, the mentioned research demonstrated that increased microbial count led to the higher production of volatile alkaline metabolites, thereby leading to the color changes of the indicator(12).

The pH sensitive dye, bromocresol green, was used by Pacquit for monitoring the fish (Cod) spoilage. They reported that after 26 h storage by changing the color of indicator, the TVC count rose to 10^8 CFU/g(5). It was suggested that using colorimetric sensors could be effective method to guarantee the freshness and estimation of best-before date, reduce margins of errors and food wastage(2, 5, 26). It was reported that due to microbial growth, the pH and volatile gases of meat (volatile amines) increased(16).

According to the findings of the current research, the total bacterial count was higher on day eight of storage compared to the permissible limit of 3×10^6, and the pH also increased during the refrigerated storage of meat. Several studies have indicated that biogenic amines such as triamines, tryptamines, putrescine, and cadaverine are associated with indicators such as TVBN, pH, and total bacterial count in chicken meat and other meat products(27).In addition, increased microbial growth results in the elevation of metabolites (e.g., carbon dioxide and volatile nitrogen compounds), which are used as the parameters of indicator quality; this finding is consistent with the previous studies in this regard.

In the present study, the first signs of chicken spoilage were detected on day eight of storage, which was indicated by the green color of the indicator, poor sensory analysis, and higher amounts in the chemical and microbiological analyses. Moreover, direct correlations were observed between the sensory analysis, bacterial count, pH, and TVBN. During the chicken meat storage, the alkaline volatile amines were gradually produced in the packaging space, thereby increasing the pH (>6) and changing the color of the indicator. These findings are in congruence with the study by Kuswandi et al. (23).

Chicken meat freshness is a highly complex subject, which depends on several factors, such as storage duration, packaging conditions, initial microbial load, and moisture(21). Therefore, the indicator described in the present study could be used as a proper and cost-efficient tool for the assessment of chicken meat spoilage in order to immediately inform consumers of the freshness of chicken (day eight).Our findings showed a significant coloration between TVC and indicator response, which enabled the real-time monitoring of chicken spoilage.

Conclusion

According to the results, the BCG indicator could be employed to represent the freshness of chicken meat. When chicken meat is spoiled, the color of the label containing the colored indicator (BCG and gelatin) also changed, thereby increasing TVBN, pH, and microbial load. The changes in TVBN, pH, and microbial load had a direct, positive correlation with the indicator color. The BCG indicator is a simple and inexpensive colorimetric method to be used by consumers in order to ascertain chicken meat freshness.

Conflicts of interest

None declared.

Acknowledgments

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