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Development of a food spoilage indicator for monitoring freshness of skinless chicken breast

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ABSTRACT

A colorimetric mixed-pH dye-based indicator with potential for the development of intelligent packaging, as a "chemical barcode" for real-time monitoring of skinless chicken breast spoilage, is described. Also investigated was the relationship between the numbers of microorganisms and the amount of volatile compounds. This on-package indicator contains two groups of pH-sensitive dyes, one of which is a mixture of bromothymol blue and methyl red, while the other is a mixture of bromothymol blue, bromocresol green and phenol red. Carbon dioxide (CO_2) was used as a spoilage metabolite because the degree of spoilage was related to the amount of increased CO_2, and which was more than the level of total volatile basic nitrogen (TVB-N) during the storage period. Characteristics of the two groups of indicator solutions were studied, as well as their response to CO_2. A kinetic approach was used to correlate the response of the indicator label to the changes in skinless chicken breast spoilage. Color changes, in terms of total color difference of a mixed-pH dye-based indicator, correlated well with CO_2 levels of skinless chicken breast. Trials on skinless chicken breast samples have verified that the indicator response correlates with microbial growth patterns, thus enabling real-time monitoring of spoilage either at various constant temperatures or with temperature fluctuation.

1. Introduction

Consumer demand for mildly preserved, minimally processed, easily prepared and ready-to-eat "fresher" foods – together with the globalization of the food business, and the logistics of distribution from processing centers – poses major challenges for food quality and safety [1,2]. There is a great interest among members of the food industry – including retailers, consumers' rights watchdogs, and food safety controlling bodies – in developing accurate, cost-effective, rapid, reliable, non-invasive and non-destructive methods or devices to evaluate real-time freshness of food products. An alternative concept to meet this requirement is the development of intelligent packaging in the form of a food spoilage indicator to monitor freshness status [3–6].

Poultry meat is a highly perishable food and usually deteriorates within 1 week of slaughter, regardless of chilled storage systems. Such spoilage is largely due to different types of microorganisms – including bacteria, such as *Pseudomonas* spp. and *Shewanella putrefaciens*, and yeasts – depending on the initial microbiological quality of the poultry carcass [7]. In the case of aerobic storage, *Pseudomonas* spp. and yeasts are the main microorganisms that prevail [8,9]. Equally important is the fact that *Pseudomonas* spp. (including the human pathogens *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, which are rarely implicated in foodborne disease outbreaks) have been linked with the spoilage of fresh poultry [10–13]. Rodriguez et al. [14] reported that the increased package CO_2 concentration caused a reduction in the growth rate of aerobic heterotrophic mesophyll bacteria (AHMB), aerobic heterotrophic psychotropic bacteria (AHPB), Enterobacteriaceae, and lactic acid bacteria (LAB), and treatment with 90% CO_2 appears promising as a method with which to increase the shelf life of ready-to-eat shredded chicken breast.

The freshness of refrigerated meat is reduced in time as a result of biochemical, physicochemical and microbiological transformations. The loss of freshness indicates that meat has started to spoil. Microorganisms with proteolytic activity can act on proteins, transforming them into smaller compounds such as free amino

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acids. The amino acids can suffer oxidative deamination, decarboxylation and desulfurization, resulting in gases such as NH_3, CO_2, and H_2S. Meat itself contains free amino acids; proteins in meat can also be broken down into amino acids by hydrolysis. Subsequently, they can be degraded partially or totally into simple compounds such as CO_2, H_2O, NH_3, and H_2S. [15]. Carbon dioxide (CO_2) is generally known to be produced during microbial growth. Another indicator of microbiological spoilage of food protein is high levels of total volatile basic nitrogen (TVB-N), e.g., ammonia, dimethylamine (DMA) and trimethylamine (TMA) [13,16].

Quantifying chemical changes could thus provide information on the degree of spoilage. A number of chemical indicators have been proposed to assess meat quality, including biogenic amines (BAs) [17], volatile bases [18], nucleotide breakdown products, and volatile acidity [3]. Consequently, these compounds can be employed as quality indicators of fresh chicken during storage. According to Smolander [16], color changes of pH dyes (e.g. bromothymol blue, bromophenol blue, bromocresol purple, methyl red, bromocresol green, methyl orange, methyl yellow, phenol red) can be employed to detect acidic/basic volatile compounds, as they display an irreversible change in visual appearance.

The objectives of this study were: (i) to investigate the relationship between the amount of volatile compounds and the numbers of microorganisms; and (ii) to develop a food spoilage indicator for monitoring the freshness of skinless chicken breast.

2. Materials and methods

2.1. Materials

Sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$) and disodium hydrogen orthophosphate (Na_2HPO_4) (Fluka Chemie, Switzerland); plate count agar (Merck, Germany); and cetrimide fucidin cephaloridine agar, streptomycin sulfate–thallous acetate–cycloheximide (actidione) agar, and violet red bile glucose agar (Oxoid, UK) were used for microbiological analyses. Filter paper (#41; Whatman, Germany), ethanol (Sigma-Aldrich, USA), bromocresol green and bromothymol blue (Ajax Finechem, Australia), and methyl red (Panreac Química, Spain) were used to prepare a dye mixture label. Food-grade methylcellulose (MC) (Methocel™; Dow Chemical, USA) was used as the carbohydrate biopolymer for coating formulations. Polyethylene glycol 400 (Carbowax™; Dow Chemical, USA) was added as the plasticizer. Double distilled and de-ionized (DI) water having almost zero conductivity was used as the solvent. Optically clear polyamide laminated with linear low-density polyethylene (nylon/LLDPE, 80-μm grade) and linear low-density polyethylene (LLDPE, 50-μm grade) films were obtained from Amcor Flexibles Bangkok Public Co. Ltd., Thailand.

2.2. Skinless chicken breast spoilage study

2.2.1. Experimental setup

Fresh skinless chicken breasts were purchased from Betagro Food Co. Ltd. and transported to the laboratory within 1 h of purchase. Samples were used in each series of experiments. First, 502.82 g of skinless chicken breast samples were aseptically placed into sterilized 1,000 mL Erlenmeyer flasks. Samples were stored at 4 and 10 °C and periodically analyzed for product quality during storage.

2.2.2. Microbial analysis

Skinless chicken breast samples were examined for levels of total aerobic bacteria, *Pseudomonas* spp., Enterobacteriaceae and *Brochothrix thermosphacta*. Duplicate samples from each treatment were aseptically opened on the sampling days; then a 25 g portion of skinless chicken breast was aseptically transferred to a sterile stomacher bag. Next, 225 mL of 0.1 M sterile sodium phosphate buffer solution (pH 7.0) was added and homogenized for 1 min by a Stomacher® 400 laboratory blender (Seward, UK). A series of decimal dilutions was carried out according to recommended microbiological protocols [19]. In order to determine total aerobic bacteria, *Pseudomonas* spp., Enterobacteriaceae and *B. thermosphacta*, 1 mL of each appropriate dilution was pour plated in duplicate on plate count agar (for total aerobic bacteria) and on violet red bile glucose agar (for Enterobacteriaceae), and 0.1 mL of each appropriate dilution was spread plated in duplicate on cetrimide fucidin cephaloridine agar (for *Pseudomonas* spp.) and streptomycin sulfate–thallous acetate–cycloheximide (actidione) agar (for *B. thermosphacta*). Total aerobic bacteria and Enterobacteriaceae plates were incubated aerobically for 2 d at 37 °C [20] and 1 d at 37 °C [21], respectively, while *Pseudomonas* spp. and *B. thermosphacta* plates were incubated for 2 d at 25 °C [22] and 2 d at 23 °C [23], respectively. Colonies were counted and reported as log CFU (colony-forming units) g^{-1}.

2.2.3. Determination of basic and acidic species

Total volatile basic nitrogen content was determined using the Conway microdiffusion assay, as described by Ng [24]. A sample (2 g) was added to 8 ml of 4% trichloroacetic acid (TCA) (w/v) and ground well. It was then left for 30 min at ambient temperature with occasional grinding, followed by filtration through Whatman #41 filter paper. The filtrate was kept at 4 °C. This filtrate, referred to as "sample extract" (1 mL), was placed in the outer ring of a Conway apparatus. The inner ring solution (1% boric acid containing the Conway indicator) was then pipetted into the inner ring. To initiate the reaction, K_2CO_3 (1 mL) was mixed with sample extract. The Conway unit was closed and incubated at 37 °C for 60 min. The inner ring solution was then titrated with 0.02 M HCl until the green color turned to pink. The concentration of TVB-N was expressed as mg N/100 g sample, as described by Ng [24].

Acidic species were analyzed by headspace gas detection, using a headspace sample of 3 mL. All measurements were carried out using a PAC CHECK® 650 EC headspace analyzer (MOCON, USA). CO_2 produced by microorganisms within the packaging was detected and displayed as a percentage value (%) over the storage period.

2.3. Indicator fabrication

2.3.1. Indicator solutions

Two groups of mixed pH-sensitive dyes were prepared. One was a mixture of bromothymol blue and methyl red, adapted from Nopwinyuwong et al. [3] (M formula), which was prepared by mixing bromothymol blue (0.04%, w/v) and methyl red (0.04%, w/v) in aqueous ethanol in a ratio of 2:3. The other was a mixture of bromothymol blue, bromocresol green and phenol red (P formula), which was prepared by mixing bromocresol green (0.04%, w/v), bromothymol blue (0.04%, w/v) and phenol red (0.04%, w/v) in aqueous ethanol in a ratio of 6:9:35.

2.3.2. Indicator coatings

Cellulose-based indicator coating solutions were prepared by dissolving methylcellulose (2.00% w/v) and hydroxypropyl methylcellulose (1.00% w/v) powders in DI water. Polyethylene glycol-400 (2%, v/v) was added to prevent brittleness. Later on, the indicator solution for each formula, including (1) M1 formula, (2) M2 formula, having a double volume of M1 formula, and (3) P formula, according to the method of Nopwinyuwong et al. [3], was added.

To obtain the desired coating solution, the mixture was homogenized at a speed of 10,000 rpm until a complete dissolution of polymer matrix had been achieved. The coating solutions were degassed in an ultrasonic water bath (Model 275D, Crest Ultrasonics Corporation, Trenton, NJ, USA) for 10 min.

2.3.3. Indicator labels

A 22.35 g of cellulose-based coating was mixed with either 1.25 mL of M1 formula, 2.5 mL of M2 formula, or 1 mL of P formula to obtain a cellulose-based indicator coating solution. Cellulose-based indicator label was cast by pouring the indicator coating solution into a flat 13 × 24 cm glass plate wrapped with an LLDPE film. The plates were dried at ambient condition for 24 h. Filter paper was used as a base layer of the indicator label to clearly observe the color changes. Finally, the label was enclosed with active breathable film.

2.4. Color changes of indicator solutions caused by CO_2

Color changes of the indicator solutions due to contact with CO_2 were studied by enclosing 2 or 3 mL of colorimetric mixed-dye–based solutions in gas-tight vials (3 mL). Carbon dioxide was diluted with nitrogen and injected into the vials with a gas-tight syringe, obtaining final CO_2 concentrations of 0–25% (v/v). Indicator solution samples were incubated at 30 °C for 30 min, and their optical spectra recorded with a T60 UV spectrophotometer (PG Instruments, UK).

2.5. Color changes of indicator labels for skinless chicken breast spoilage trial

Skinless chicken breast samples (134.59 g) were aseptically placed into sterilized 267.67 mL PE trays and then heat-sealed with nylon/LLDPE film. Each type (M1, M2 and P formulas) of indicator label was enclosed in each PE tray. The samples were stored at 4 and 10 °C, and periodically analyzed for product quality in terms of basic and acidic species during storage. Color change of the colorimetric mixed-dye–based indicator was measured instrumentally with a Konica Minolta CR-400 spectrophotometer, using L, a, b values to describe the color of the indicator. The index describing the total color difference (TCD) was suggested by Hunt [25]. Color change (ΔE) was calculated by Eq. (1):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (1)$$

In addition, a kinetic approach was carried out to allow the correlation of the response of the indicator to the freshness of skinless chicken breast during storage at different temperatures.

3. Results and discussion

3.1. Chemical and microbiological changes in skinless chicken breast

Total volatile basic nitrogen and carbon dioxide (CO_2) were markedly detected in skinless chicken breast, and their levels increased with storage time (Fig. 1). At lower temperature, more TVB-N and CO_2 are adsorbed into the food matrix, while at higher temperature less are adsorbed. The corresponding vapor pressure of gaseous species is very high. Generally, it was found that the vapor pressure of TVB-N and CO_2 increased more and more rapidly with rising temperature. Moreover, the dynamic changes in TVB-N and CO_2 levels were observed, which could be related to the growth of microorganisms (Fig. 2). Proliferation of microflora contributed to spoilage changes, as seen by increased TVB-N and CO_2 levels.

Initially, specific spoilage organisms (SSO) are present in low quantities and constitute only a minor part of the natural microflora. During storage, SSO generally grow faster than the remaining microflora and produce the metabolites responsible for off-odors and off-flavors, and finally cause sensory rejection. The cell concentration of SSO at rejection may be called the "minimal spoilage level," and the concentration of the metabolites that correspond to spoilage can be used as an objective chemical spoilage index (CSI) [26]. *Pseudomonas* spp., which were expected to be SSO, were able to continuously grow under these conditions, faster than the total aerobic bacterial counts of Enterobacteriaceae and *B. thermosphacta* (Fig. 3). This resulted in 4.80 and 6.00 log CFU g^{-1} of total aerobic bacteria, 7.00 and 7.00 log CFU g^{-1} of *Pseudomonas* spp., 4.20 and 5.78 log CFU g^{-1} of Enterobacteriaceae, and 5.87 and 6.58 log CFU g^{-1} of *B. thermosphacta*, stored at 4 and 10 °C for 6.12 and 2.78 d, respectively, which did not exceed the limit of acceptability at the end of storage. The limit of acceptability was based on the onset of food spoilage, which was considered to be 7.00 log CFU g^{-1} of microorganisms in fresh poultry [27,28]. Thus, shelf lives of fresh chicken, stored at 4 and 10 °C, were approximately 6.12 d and 2.78 d, respectively. This is comparable to the study by Khanjari et al. [29], which found that the shelf life of chicken breast fillets stored at 4 °C was 6 d, and the study by Kuswandi et al. [13], which found that the shelf lives of broiler chicken cuts stored at 4 and 28 °C were 7 d and 8 h, respectively.

A clear correspondence was found between the microbiological quality of fresh skinless chicken breast (as a protein-based material) and the level of metabolites. TVB-N and CO_2 formation took place evenly during the storage period; however, their formation was dependent upon temperature. Higher storage temperature had a more conspicuous effect on the formation of TVB-N and CO_2, which accumulated especially at the end of the storage period. (Fig. 1). This correlation of TVB-N is in agreement with the findings of Balamatsia et al. [28] and Rokka et al. [30], who reported that TVB-N or the total amount of biogenic amines could be employed as potential chemical indicators in monitoring the microbial quality of fresh chicken meat during chill storage under aerobic and modified atmosphere packaging (MAP) conditions. The levels of TVB-N as an indicator of skinless chicken breast spoilage were approximately 18 and 17 mg N per 100 g sample when stored at 4 and 10 °C, respectively. These results were comparatively lower than the TVB-N values in the findings of Kuswandi et al. [13], although in both studies an increase in TVB-N resulted in a decrease in freshness of skinless chicken breast. A correlation between CO_2 concentration and the growth of microorganisms was found in studies by Nopwinyuwong et al. [3] and Fu et al. [31] on golden drop (an intermediate-moisture dessert) and beef rib eye steaks which were packaged aseptically in air packaging and under modified atmosphere packaging (MAP), respectively. In the present study, there was a comparatively greater increase in CO_2 than TVB-N during the storage period, as reflected by a higher slope trend for changes in CO_2 (Fig. 2). For this reason, and from the standpoint of indicator sensitivity, CO_2 was used as a spoilage metabolite of skinless chicken breast.

3.2. Color changes of indicators

When CO_2 was added to vials containing colorimetric mixed-dye–based indicator solution (P formula), a visual color change of the solution from violet to yellow was detected. The most remarkable change in the absorption spectra after 30 min of reaction time took place at high absorption peaks in the wavelength range of 435–565 nm (Fig. 4a). Bromocresol green, which shifts from basic form (blue-green, pH 5.4) to acidic form (yellow, pH 3.8), results in maximum lambda (λ_{max}) shifting from 615–620

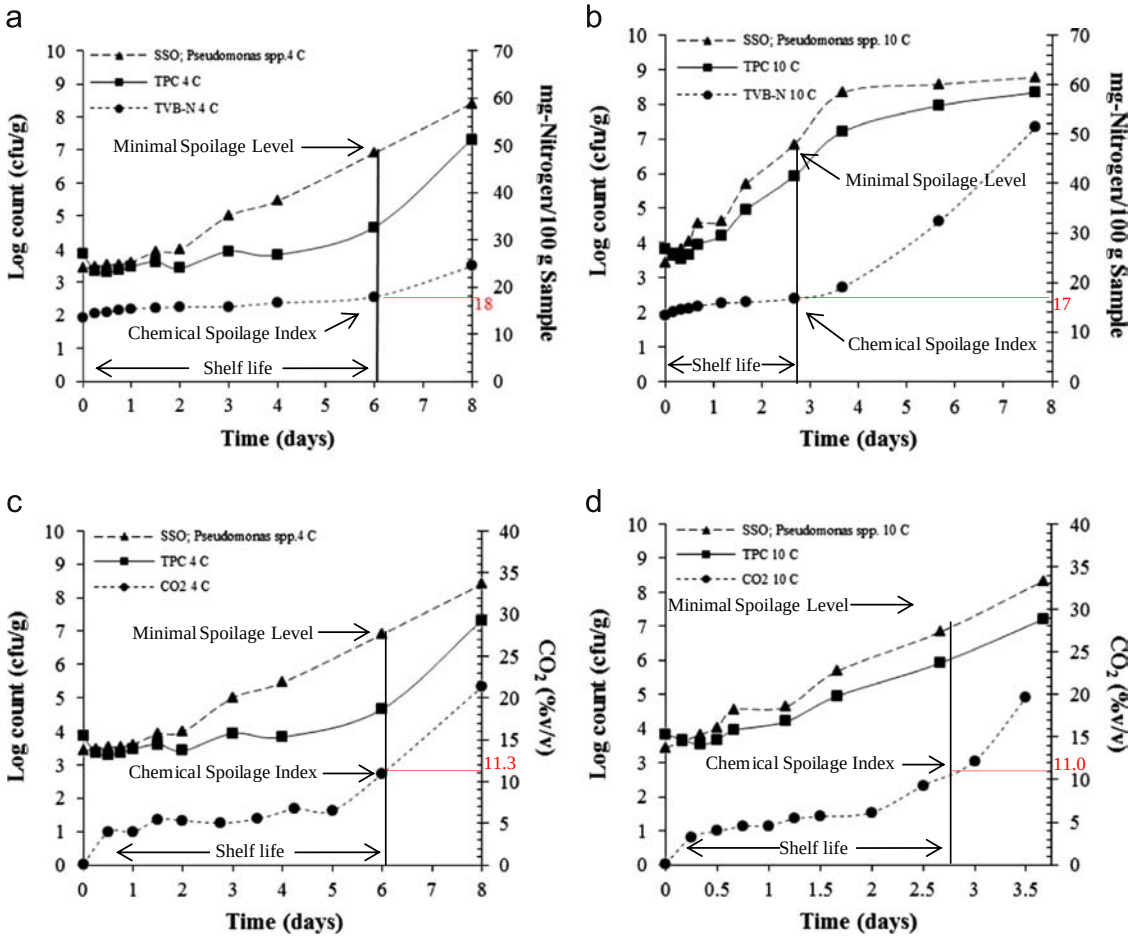

Fig. 1. Changes in metabolites (TVB-N and CO_2) and microbial counts of skinless chicken breast stored at 4 °C (a, c) and 10 °C (b, d).

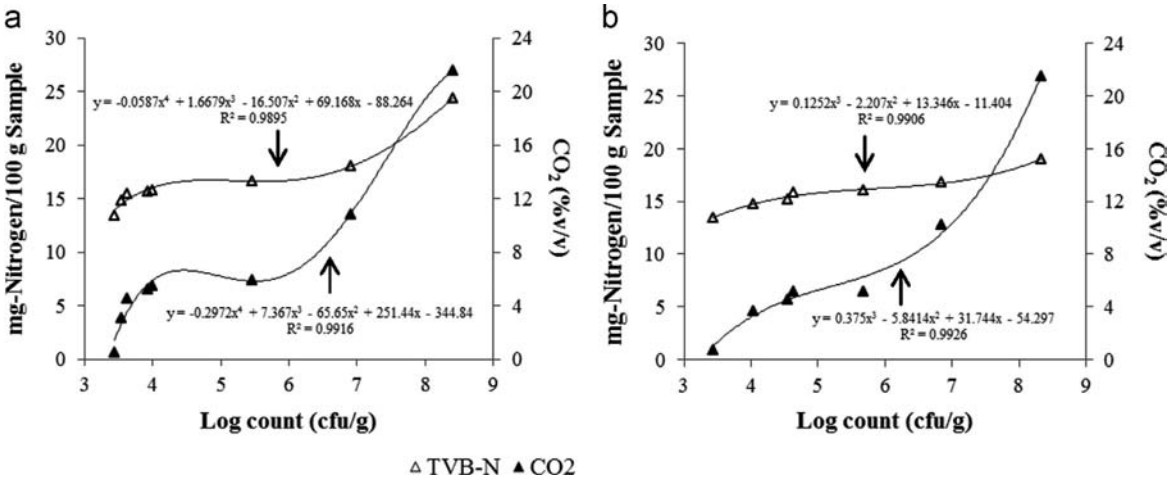

Fig. 2. Correlation between *Pseudomonas* spp. with TVB-N and CO_2 of skinless chicken breast stored at 4 °C (a) and 10 °C (b).

to 430–435 nm. Bromothymol blue, which shifts from basic form (blue, pH 7.6) to acidic form (yellow, pH 5.8), results in maximum lambda (λ_{max}) shifting from 615–620 nm to 430–435 nm. Phenol red, which shifts from basic form (red, pH 8.4) to acidic form (yellow, pH 6.8), results in maximum lambda (λ_{max}) shifting from 558–562 nm to 430–435 nm [32]. As a mixed-dye–based indicator, violet changed to yellow in relation to a shift in the absorption peak from 558–562 to 430–435 nm when exposed to levels of CO_2 from 0% (v/v) to 25% (v/v). For M1 and M2 formula indicator solutions, a visual color change from bright light green to bright red was detected, in accordance with Nopwinyuwong et al. [3] (Fig. 4b). This vivid color spectrum of mixed-dye indicator solution is in agreement with Wallach [33], who reported that a mixed indicator could enhance an expansion of the range of color change, as compared with a single indicator. Fig. 5 shows the visual color changes of colorimetric mixed-dye–based indicator labels. It was found that P type, M1 type and M2 type indicator labels showed, respectively, a clear spectrum from purple to green, from bright

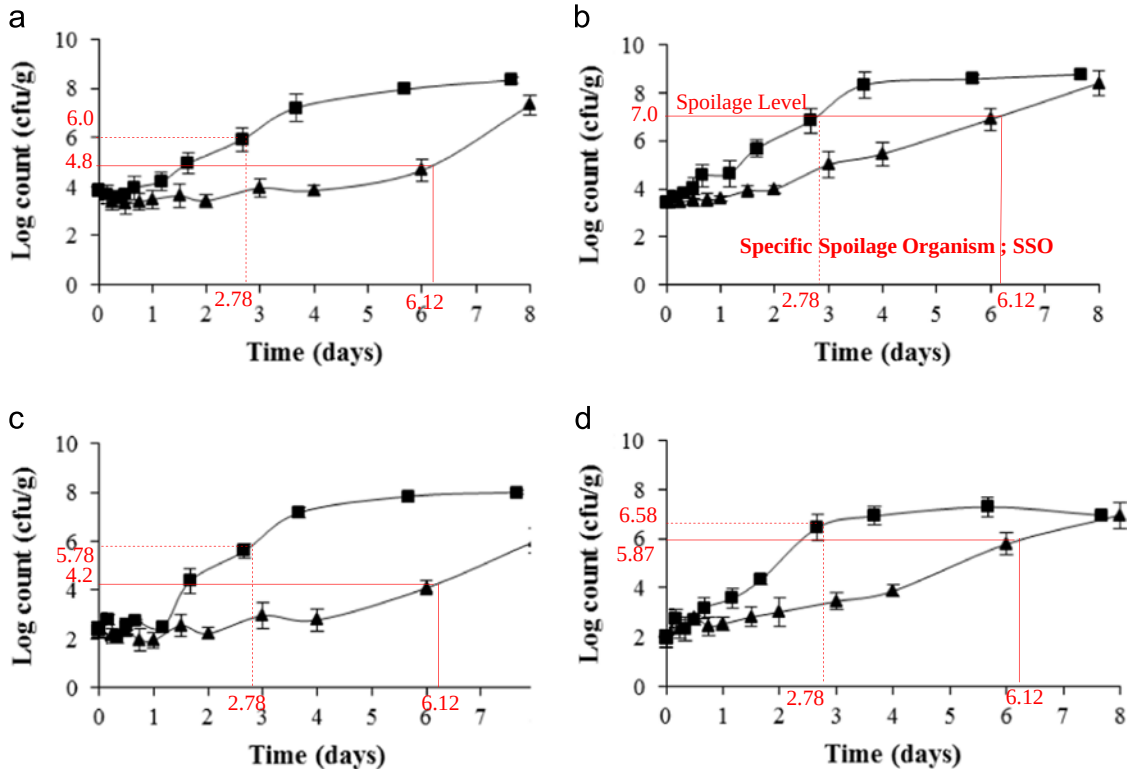

Fig. 3. Changes in microbial counts of skinless chicken breast stored at 4 °C (▲) and 10 °C (■). (a) Total aerobic bacterial counts. (b) Pseudomonas sPP. (c) Enterobacteriaceae. (d) Brochothrix thermosphacta.

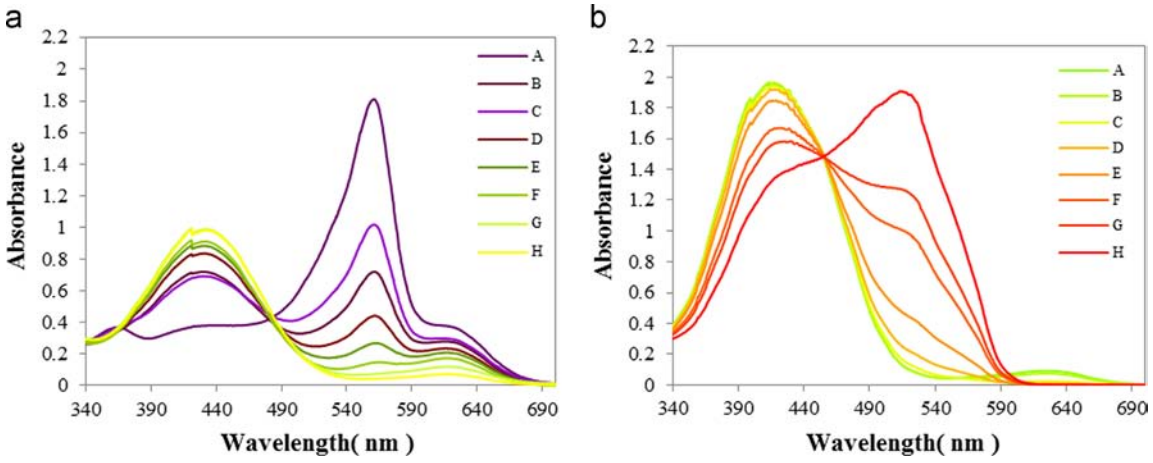

Fig. 4. Absorption spectra of mixed-dye–based indicator solutions: (a) P formula and (b) M formula. Carbon dioxide (%, v/v): *A*=0%, *B*=2.5%, *C*=5.0%, *D*=7.5%, *E*=10.0%, *F*=15.0%, *G*=20.0% and *H*=25%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

light green to orange-red, and from dark green to dark orange when exposed to a level of CO_2 in a range of 0–23% (v/v). The main purpose of applying colorimetric mixed-dye–based indicators to food packaging is to easily and reliably monitor the level of food spoilage of packaged food products in a non-destructive manner during distribution and retail sale. The levels of food spoilage via the formation of CO_2 were correlated with the total color difference values of each indicator label.

3.3. *Color changes of indicator labels during skinless chicken breast spoilage trial*

Fig. 6. shows the change in CO_2 level monitored by colorimetric mixed-dye–based indicator labels (M1, M2 and P types) in skinless chicken breast at 4 and 10 °C. These findings showed that the minimal spoilage level of skinless chicken breast occurred on days 6.12 and 2.78 when stored at 4 and 10 °C, respectively. When exposed to CO_2 during storage, the M1 and M2 types showed spectral ranges from bright light green to yellow, and from bright light green to orange-yellow, respectively, while the P type showed a spectrum from violet to green. Similar to the findings of Kuswandi et al. [13], the methyl red–based sensor changed color from red to yellow after 7 d and after 8 h at chilled and room temperatures, respectively, corresponding to increased TVB-N levels. Carbon dioxide from the headspace dissolved in the filter layer, which is hydrophilic material, forming carbonic acid in the presence of moisture. Carbonic acid is diprotic, having two hydrogen atoms which may dissociate from the parent molecule to form hydrogen ions (H^+) and bicarbonate ions (HCO^{3-}) with pK_a of 6.36 at 25 °C [34]. Then, as a proton, a hydrogen ion combines with

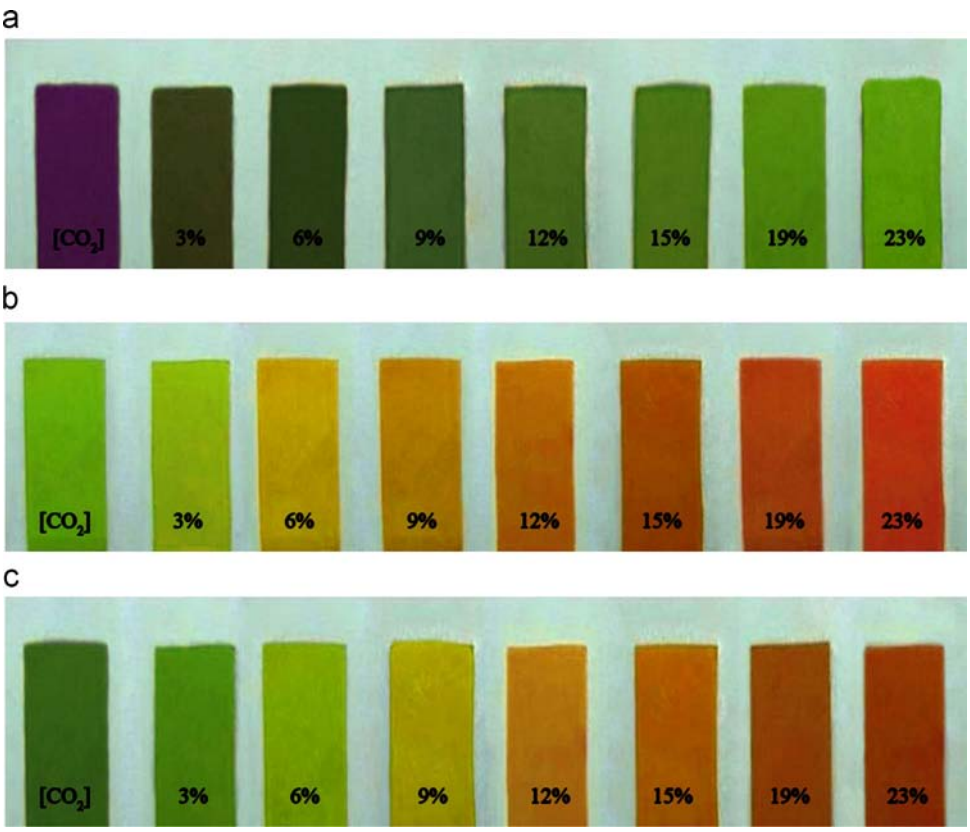

Fig. 5. Changes in color of indicator labels in response to CO_2, by type: (a) P, (b) M1, and (c) M2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

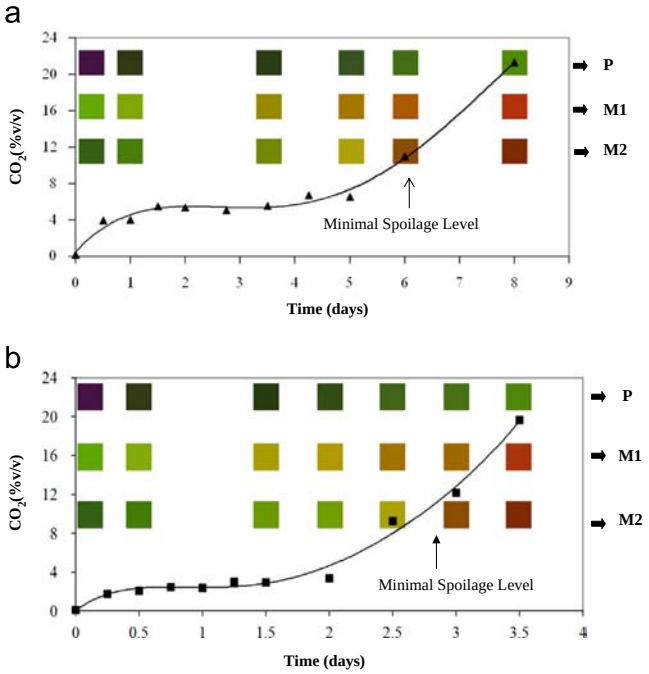

Fig. 6. Changes in CO_2 level in skinless chicken breast at 4 °C (a) and 10 °C (b), with indicator labels (M1, M2 and P types) showing color changes over time. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

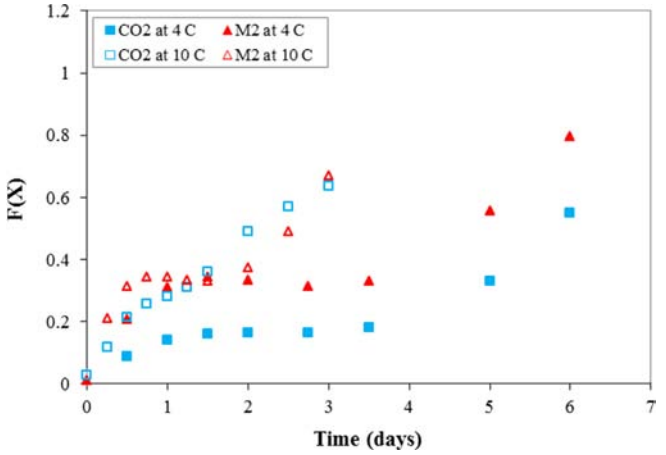

Fig. 7. Plot of response function $F(X)$ with time for CO_2 levels in skinless chicken breast at (■) 4 °C and (□) 10 ºC; and for total color difference in indicator labels at (▲) 4 °C and (△) 10 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

a water molecule to form a hydronium ion, (H_3O^+). Hydronium ions react with the basic form (In^-) of the indicator label, resulting in an acid form (HIn) which in turn produces a color change of the indicator label [32]. Skinless chicken breast is highly nutritious (protein content ~23.60%) and possesses a slightly acid pH and high moisture content (75.61%) [35], which therefore permits growth of a wide range of microorganisms. The pattern of CO_2 formation has been found to be similar to the general pattern of the chemical spoilage index [36].

The changes of CO_2 as a result of skinless chicken breast spoilage can be modeled theoretically, in accordance with Hong and Park [37]. The CO_2 level of skinless chicken breast consistently changed over time, as represented by a sigmoidal curve. Polynomial functions give the lines of this curve, represented by Eq. (2):

$$X = a_n x^n + a_{n-1} x^{n-1} + \cdots + a_2 x^2 + a_1 x + a_0 = (CO_2 - CO_{2i})/CO_{2m} \quad (2)$$

Table 1
Response rate constants and coefficients of determination[a] for CO_2 of skinless chicken breast and for total color difference (TCD) of indicator labels at different temperatures.

Temperature (°C)	CO_2		TCD					
			M1		M2		P2	
	k (day^{-1})	r^2	k (day^{-1})	r^2	k (day^{-1})	r^2	k (day^{-1})	r^2
4	0.1025	0.8244	0.0778	0.9136	0.0925	0.8074	0.0718	0.7199
10	0.1946	0.9801	0.2199	0.9605	0.1964	0.8829	0.1882	0.8125

[a] Significance level of $P<0.05$.

where X is a normalized CO_2 value, CO_{2i} is the initial CO_2 value at $t=0$, and CO_{2m} is the maximum CO_2 value measured. At 4 °C, the function with a coefficient of determination (r^2) of 0.9909 is given by Eq. (3):

$$X = 0.0002x^5 - 0.0041x^4 + 0.0433x^3 - 0.192x^2 + 0.3645x \quad (3)$$

At 10 °C, the function with r^2 of 0.9964 is given by Eq. (4):

$$X = 0.0192x^5 - 0.1742x^4 + 0.6204x^3 - 1.0007x^2 + 0.7745x \quad (4)$$

The above equation can be rearranged to the desirable form in Eq. (5):

$$F(X) = [\ln\{1/(1-X)\}]^{1/2} = kt \quad (5)$$

where k is the reaction or response rate constant, and t is time.

Plotting the function $F(X)$, given by the logarithmic expression in the above equation, versus time gives two straight lines of different slopes at each temperature (Fig. 7). The rate constants and the coefficients of determination for CO_2 levels of skinless chicken breast at different temperatures are listed in Table 1.

The total color difference (TCD) values also changed continuously and consistently with the response of the indicator labels. TCD values gradually increased with time. The final TCD values were 16.38–26.00. It is generally known that TCD values greater than 5 can be easily detected by the unaided eye, and TCD greater than 12 indicates a completely different color space [38]. The changes in TCD of the label showed a zero-order behavior, even though at a different rate. Therefore, a kinetic approach could be used to model the measurable TCD change of the indicator label. A normalized TCD value, X=TCD, was used as the variable. The indicator response X when plotted against time gives straight lines (Fig. 7) if $F(X)=kt$ is defined as the response function. The rate constants and coefficients of determination for TCD of indicator labels (M1, M2 and P types) at different temperatures are given in Table 1. It was found that the rate constant of TCD in the M2 type was close to the rate constant of CO_2 at 4 and 10 °C, compared with the other label types.

If the food spoilage indicator and the food product quality deterioration reactions have similar temperature dependence, the indicator can be accurately used to monitor the freshness of food products. Examples of packaged skinless chicken breast with food spoilage indicator labels (M1, M2 and P types) are shown in Fig. 8. This attempt is to initiatively develop a mixed colorimetric indicator label for monitoring freshness of skinless chicken breast being air-packed. It will include air packaging, but not limited to modified atmosphere packaging (MAP). In a case of MAP with high level of CO_2 of about 25%, this mixed colorimetric indicator label is required to adjust a formula via NaOH solution in order to be equivalent to the starting concentration of CO_2 (25%) in the headspace of the package, in turn, the color of indicator type P, M1, and M2 will be reddish purple, deep green, and greenish blue, respectively. After modified atmosphere packaging, these indicators will firstly react with 25% CO_2, resulting in purple, bright green and deep green for type P, M1 and M2, respectively. Later on, these indicators will react with CO_2 produced by spoilage microorganisms, leading to a change in color, as represented in Fig. 5.

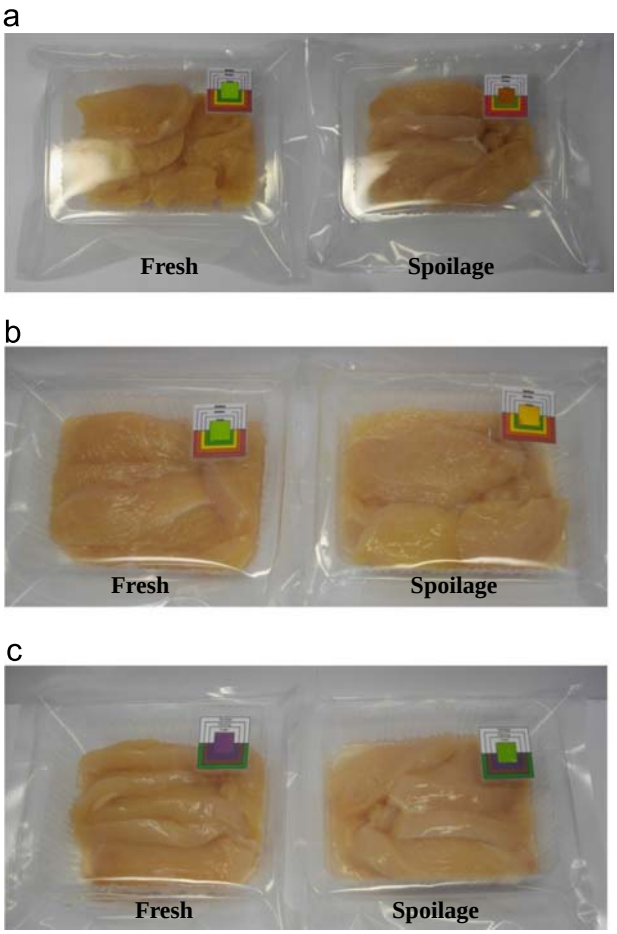

Fig. 8. Packaged skinless chicken breast with food spoilage indicator labels. (a) M1 type: green=fresh, yellow=spoilage; (b) M2 type: green=fresh, orange-yellow=spoilage; and (c) P type: violet=fresh, green=spoilage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

4. Conclusions

The results presented in this study indicate that when compared with other dye mixtures, a mixture of bromothymol blue and methyl red (M2 type) provided the fastest and most sensitive detection of spoilage metabolites in skinless chicken breast, which can be achieved by a non-invasive colorimetric method. The indicator response was found to correlate with microbial growth patterns in skinless chicken breast samples, therefore enabling real-time monitoring of spoilage. In addition, this study also assessed the indicator response at refrigerated temperature, as

microbial population and microbial activity are both temperature-dependent. This colorimetric mixed-dye–based food spoilage indicator allows the food product to have an effective shelf life by permitting dynamic freshness to be monitored visually alongside the best-before date, consequently decreasing margins of error. The enhanced guarantee of food product safety is of primary interest to consumers. In addition, product confidence is of great importance to manufacturers and retailers in order to protect their brand value by preventing customer dissatisfaction.

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