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Development of a novel colorimetric indicator label for monitoring freshness of intermediate-moisture dessert spoilage

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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 intermediate-moisture dessert spoilage, is described. This on-package indicator contains mixed pH-sensitive dyes, bromothymol blue and methyl red, that respond through visible color change to carbon dioxide (CO_2) as a spoilage metabolite. Both indicator solution and indicator label characteristics 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 intermediate-moisture dessert spoilage. Color changes, in terms of total color difference of a mixed pH dye-based indicator, correlated well with CO_2 levels of intermediate-moisture dessert. Trials on golden drop have verified that the indicator response correlates with microbial growth patterns in dessert samples, thus enabling the 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 – pose major challenges for food quality and safety [1,2]. A reduction in shelf life of foods as a result of microbial contamination and an increase in the risk of food-borne illness are driving forces for innovative means to monitor microbial growth in the foods while enhancing food safety. Moreover, food traceability is now a legal requirement, especially in the European Union. This establishes a chain of responsibility throughout the entire food supply chain. Consequently there is great interest among the food industry, 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. In addition to the development of a food spoilage indicator, the detection of chemical changes associated with microbial growth in food products can offer an alternative to sensory and microbiological analyses which are often costly and time-consuming [3].

Intelligent packaging can be defined as a mode of packaging that is capable of carrying out intelligent functions (such as detecting, sensing, recording, tracing, communicating, and applying scientific logic) to facilitate decision making to extend shelf life, enhance safety, improve quality, provide information, and warn about possible problems [4]. As a subclass of intelligent packaging, a food spoilage indicator is a packaging system (or material) which uses metabolites as "information" to monitor the status of food spoilage.

The amount of published work on food spoilage indicators is still limited. Some trials, however, have constructed indicators for the volatile compounds produced in microbial spoilage. Smolander et al. [5] developed myoglobin-based indicators for modified-atmosphere-packed poultry meat on the basis of the presence of hydrogen sulphide (H_2S). Pacquit et al. [6] and Pacquit et al. [7] also developed a colorimetric dye-based sensor and indicator for monitoring fish spoilage on the basis of the presence of total volatile basic nitrogen (TVB-N).

A principal prerequisite in the development of food spoilage indicators is knowledge of the quality-indicating metabolites. In systems such as air packaging or nitrogen-flushing, aerobic and facultatively anaerobic microorganisms are able to grow during storage of the food products. Organic acids such as lactic acid and acetic acid are the major compounds that play a role in glucose fermentation by lactic acid bacteria. In addition to lactic and acetic acids, ethanol is another major end product of the fermentative metabolism of lactic acid bacteria. Carbon dioxide (CO_2) is generally known to be produced during microbial growth. It is well known that high levels of basic volatile nitrogen compounds like ammo-

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nia, dimethylamine and trimethylamine indicate microbiological spoilage of food protein [8].

Intermediate-moisture foods (IMF) are characterized by a moisture content of around 15–50% and water activity (a_w) between 0.60 and 0.85 [9]. A product such as Thai dessert is preserved by the addition of sugar. The spoilage of intermediate-moisture desserts is caused primarily by osmotolerant yeasts. As a typical intermediate-moisture Thai dessert, "golden drop" (in Thai called *thong yod*), derived from a Portuguese egg-based dessert [10], is frequently served on auspicious occasions such as marriage ceremonies or ordination ceremonies [11]. Due to its great taste, aroma and appearance, it is popular among Thai people. Golden drop is also recognized as a premium dessert. Presently, golden drop production is one of the small and medium enterprises (SMEs) in Thailand. Therefore, the shelf life of golden drop is an important concern in relation to its distribution in the marketplace.

Commercial Thai dessert products that are mostly packaged in flexible film pouches or rigid plastic trays with lidding film have greatly increased their share of the Thai food market. Without direct tasting or smelling, at the time of a retail sale it is very difficult to judge the freshness of individually packaged Thai dessert products. Most Thai desserts, due to their relatively high a_w, are easily deteriorated by microorganisms. These undesirable changes in color, flavor, odor and texture during distribution and storage make them unacceptable to the consumer [12]. Different odors are produced as metabolic products by different microorganisms (i.e. bacteria, yeast and mold). Carbohydrates, the main ingredient in Thai desserts, are continuously broken down by microorganisms into a variety of low-molecular-weight molecules such as organic acids, ethanol, CO_2, and sometimes aldehydes or ketone. Consequently, these compounds can be employed as quality indicators of Thai desserts during storage. According to Smolander [8], color change of pH dyes can be employed to detect acidic/basic volatile compounds, and display an irreversible change in visual appearance. The objectives of this study are to investigate the relationship between the amount of volatile compounds and the numbers of microorganisms, and to develop a food spoilage indicator for monitoring the freshness of intermediate-moisture Thai desserts.

2. Materials and methods

2.1. Materials

Ethanol (Sigma–Aldrich, USA), sodium dihydrogen orthophosphate ($NaH_2PO_4{\cdot}2H_2O$) (Fluka Chemie, Switzerland), disodium hydrogen orthophosphate (Na_2HPO_4) (Fluka Chemie, Switzerland), sodium chloride (NaCl) (Ajax Finechem, Australia), potato dextrose agar (PDA) (Hi-Media, India), plate count agar (Hi-Media, India), phenolphthalein (Asia Pacific Specialty Chemicals, Australia) streptomycin (M&H Manufacturing, Thailand), and glucose (Boots Manufacturing, Thailand) were used for microbiological analyses. Bromothymol blue (Ajax Finechem, Australia) and methyl red (Panreac Quimica, Spain) were used to prepare a dye mixture. Food-grade methylcellulose (MC) (Methocel®, Dow Chemical, USA) was used as the carbohydrate biopolymer for coating formulations. Polyethylene glycol-400 (Carbowax, Union Carbide, USA) was added as a plasticizer. Optically clear polyamide laminated with linear low-density polyethylene (nylon/LLDPE, 80-μm grade) films were obtained from Alcan Packaging Strongpack, Thailand.

2.2. Intermediate-moisture Thai dessert spoilage study: Golden drop case

2.2.1. Experimental setup

Fresh golden drop was ordered from a local dessert shop and transported to the laboratory within 1 h of the finished process. Samples were used in each series of experiments. First, 500 g of golden drop samples were aseptically placed into sterilized 1000 mL Erlenmeyer flasks. Samples were stored at 10 and 25 °C and periodically analyzed for product quality during storage.

2.2.2. Determination of acidic specie

Acidic specie was analyzed by headspace gas chromatography (GC) [13], using a headspace sample of 3 mL. All measurements were carried out using a HP-6890 capillary gas chromatograph equipped with a thermal conductivity detector (Hewlett–Packard, now Agilent Technologies, USA). GC conditions were: a porous-wall, large-diameter capillary column coated with divinylbenzene homopolymer (GS-Q, Agilent Technologies, USA), which was operated at 30 °C; and a carrier gas (helium) flow rate of 5 mL/min. Headspace injection operating conditions were: oven temperature of 60 °C, and thermal conductivity detector temperature of 200 °C.

2.2.3. Microbial analysis

Golden drop samples were examined for levels of mesophilic bacteria and yeast/mold. Triplicate samples from each treatment were aseptically opened on the sampling days; then a 25 g portion of golden drop 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 [14]. In order to determine mesophilic bacteria and yeast/mold counts, 1 mL of each appropriate dilution was pour-plated in duplicate on plate count agar (for bacteria) and potato dextrose agar (for yeast/mold). Mesophilic bacteria plates were incubated aerobically for 2 d at 37 °C, while yeast/mold plates were incubated for 5 d at 30 °C. Due to the high sugar content of golden drop, osmophilic yeast was also analyzed using PDA supplemented with glucose (55%, w/v). Colonies were counted and reported as log CFU (colony forming units) g^{-1}.

2.3. Indicator fabrication

2.3.1. Indicator solution

Indicator solution with a concentration of 1% (v/v) in ethanol (50%, v/v) was prepared by mixing bromothymol blue (0.1%, w/v) in ethanol (50%, v/v) and methyl red (0.1%, w/v) in ethanol (50%, v/v) in a ratio of 2:3.

2.3.2. Indicator coating

Indicator coating, with a concentration of 0.5% (v/v), contained: a binder, such as methylcellulose (3%, w/v); a plasticizer, such as polyethylene glycol-400 (1%, w/v) in distilled water; and indicator solution with a concentration of 1% (v/v) in ethanol (50%, v/v). To obtain the desired coating solution, the mixture was homogenized at a speed of 10,000 rpm until complete dissolution of the binder had been achieved. This solution was then degassed in a CREST 275D ultrasonic water bath for 10 min.

2.3.3. Indicator label

Indicator label was obtained by casting indicator coating onto nylon/LLDPE film to obtain a thickness of 145 μm; label was then dried at room temperature for 24 h. After that, a filter layer of cellulose-based coating (0.75%, w/v) was coated onto the surface of the indicator label to obtain a thickness of 147 μm; label was then dried at room temperature for 2 h.

2.4. Color changes of indicator caused by CO_2

Color changes of the indicator solution due to contact with CO_2 were studied by enclosing 2 or 3 mL of colorimetric mixed-dye-

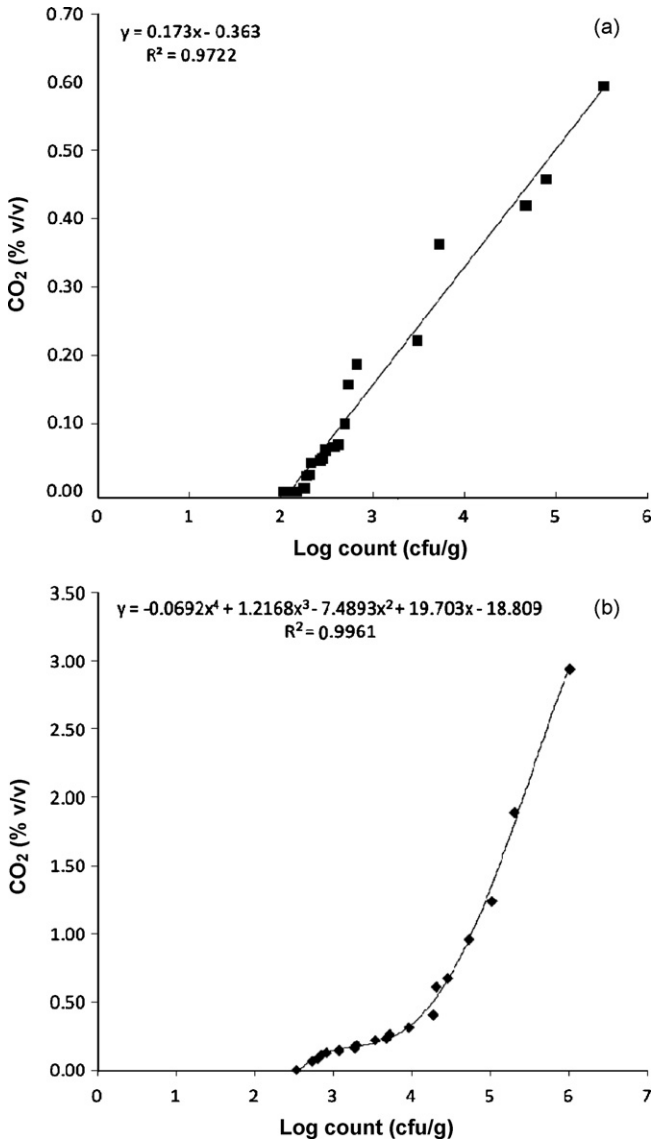

Fig. 1. Correlation between mesophilic bacteria and CO_2 of golden drop stored at (a) 10 °C and (b) 25 °C.

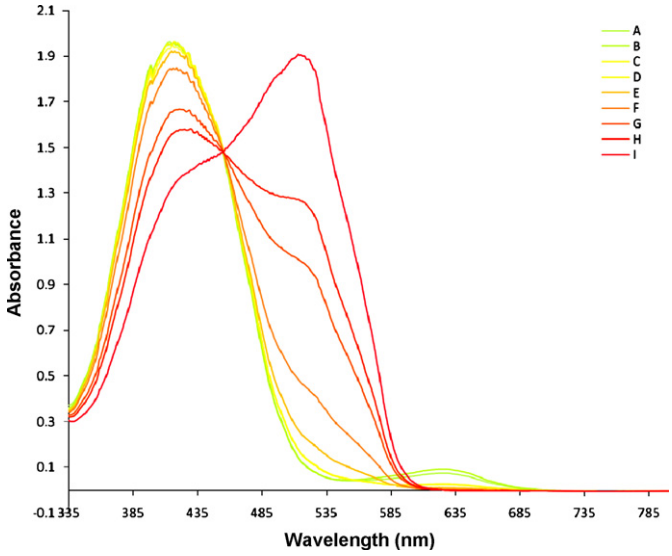

Fig. 2. Absorption spectra of indicator solution. Carbon dioxide (%, v/v): A = 0%, B = 0.1%, C = 0.5%, D = 1.0%, E = 1.5%, F = 2.0%, G = 2.5%, H = 3.0% and I = 3.5%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

based solution 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 0–3.5% (v/v). Indicator solution samples were incubated at 30 °C for 30 min, and their optical spectrum recorded with a T60 UV spectrophotometer (PG Instruments, UK).

Color changes of indicator label due to contact with CO_2 were studied by enclosing indicator labels in gas-tight 250 mL Erlenmeyer flasks. Carbon dioxide was diluted with nitrogen and injected into the flasks with a gas-tight syringe, obtaining CO_2 concentrations of 0–2.5% (v/v). The color change of the colorimetric mixed-dye-based indicator was evaluated visually and measured instrumentally with a Minolta CM-3500d 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 [15]. Color change (ΔE) was calculated with formula (1):

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

2.5. Color changes of indicator label for intermediate-moisture Thai dessert spoilage trial: Golden drop case

Five hundred grams of golden drop samples were aseptically placed into sterilized 1000 mL Erlenmeyer flasks. An indicator label was enclosed in each gas-tight flask. The samples were stored at 10 and 25 °C, and periodically analyzed for product quality in terms of acidic species during storage. The color change of the colorimetric mixed-dye-based indicator was measured instrumentally using *L*, *a*, *b* values to describe the color of the indicator. In addition, a kinetic approach was carried out to allow the correlation of the response of the indicator to the freshness of golden drop during storage at different temperatures.

3. Results and discussion

3.1. Chemical and microbiological changes in golden drop

Carbon dioxide was markedly detected in golden drop, and its level increased with storage time (Tables 1 and 2). At lower temperature, more CO_2 is adsorbed into the food matrix, while at higher temperature less is adsorbed. For gaseous specie, its corresponding vapor pressure is very high. Generally, it was found that the vapor pressure of CO_2 increases more and more rapidly with rise of temperature [16]. Carbon dioxide tends to be released from the food matrix into the headspace; thus, a quantification analysis can be performed by measuring headspace CO_2 content using GC. Since the GC column is operating at 30 °C, any water vapor entering the column will condense, which may affect the column separation performance. Thus, the temperature of 60 °C was chosen as a compromise between reducing CO_2 solubility (high temperature) and minimizing water vapor in the headspace (low temperature) [13].

Moreover, a dynamic change in CO_2 level was observed, which could be related to the growth of microorganisms (Fig. 1). Proliferation of the microflora contributing to spoilage changes as seen by increased CO_2 level coincided with the detection of acetic acid and ethanol (data not shown). Spoilage by yeasts or molds caused serious deterioration, and produced slime on day 32 and day 7.5 and visible growth on surfaces on day 34 and day 8 during storage at 10 and 25 °C, respectively. The high sugar content (20%, w/w) and, in

Table 1
Changes in CO_2 and microbial counts of golden drop during storage at 10 °C.

Time (days)	Mesophilic bacteria[a] (log CFU g^{-1})	Yeast and mold (log CFU g^{-1})	Osmophilic yeast (log CFU g^{-1})	CO_2 (%, v/v)
0	2.03 ± 0.08	0.00 ± 0.09	1.52 ± 0.07	0.000 ± 0.001
2	2.07 ± 0.07	0.00 ± 0.08	1.80 ± 0.07	0.000 ± 0.001
4	2.18 ± 0.08	1.00 ± 0.08	1.98 ± 0.07	0.000 ± 0.001
6	2.26 ± 0.08	1.22 ± 0.08	2.12 ± 0.07	0.005 ± 0.002
8	2.28 ± 0.09	1.48 ± 0.08	2.26 ± 0.08	0.023 ± 0.003
10	2.32 ± 0.07	1.53 ± 0.08	2.30 ± 0.08	0.025 ± 0.005
12	2.33 ± 0.10	1.82 ± 0.08	2.37 ± 0.08	0.042 ± 0.003
14	2.43 ± 0.08	2.00 ± 0.08	2.52 ± 0.08	0.045 ± 0.007
16	2.46 ± 0.08	2.34 ± 0.09	2.57 ± 0.08	0.049 ± 0.005
18	2.49 ± 0.07	2.55 ± 0.08	2.60 ± 0.07	0.061 ± 0.005
20	2.58 ± 0.07	2.67 ± 0.08	2.69 ± 0.07	0.066 ± 0.004
22	2.63 ± 0.08	2.72 ± 0.08	2.70 ± 0.08	0.069 ± 0.003
24	2.70 ± 0.10	2.83 ± 0.08	2.75 ± 0.07	0.099 ± 0.006
26	2.74 ± 0.08	2.95 ± 0.08	2.80 ± 0.07	0.157 ± 0.007
28	2.83 ± 0.07	3.36 ± 0.08	3.04 ± 0.08	0.187 ± 0.006
30	3.50 ± 0.10	3.73 ± 0.08	3.82 ± 0.08	0.221 ± 0.005
32	3.73 ± 0.07	4.44 ± 0.09	4.54 ± 0.07	0.363 ± 0.005
34	4.67 ± 0.09	4.94 ± 0.08	4.99 ± 0.08	0.419 ± 0.005
36	4.90 ± 0.09	5.56 ± 0.09	5.49 ± 0.08	0.459 ± 0.006
38	5.52 ± 0.09	5.90 ± 0.08	5.96 ± 0.07	0.595 ± 0.004

[a] Mean ± standard deviation of triplicate determinations of two batches.

turn, lower water activity (a_w = 0.85), had an especially detrimental effect on bacteria; while yeast – especially osmophilic yeasts (e.g. *Saccharomyces mellis* and *Saccharomycess rouxii*), which were expected to be a specific spoilage organism (SSO) – and mold were able to continuously grow under these conditions. Initially, 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, off-flavors or slime, 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) [17].

This resulted in 2.58 and 3.54 log CFU g^{-1} of total aerobic bacteria, and in 2.67 and 2.59 log CFU g^{-1} of total yeast/mold, stored at 10 and 25 °C for 20 and 4 d, respectively, which did not exceeded 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 6.00 and 2.70 log CFU g^{-1} of total aerobic bacteria and yeasts/molds, respectively [18]. Thus, shelf lives of golden drop, stored at 10 and 25 °C, were 20 and 4 d, respectively. This is comparable to the study by Mongkonwarawan [19], which found that shelf lives of golden drop, stored at 7 and 30 °C, were less than 21 d and less than 3 d, respectively.

A clear correspondence was found between the microbiological quality of golden drop (carbohydrate-based) and the level of metabolites. Carbon dioxide formation took place evenly during the storage period; however, its formation was dependent upon temperature. Higher storage temperature had a more conspicuous effect on the formation of CO_2, which accumulated especially at the end of the storage period (Fig. 1). This correlation is in agreement with the findings of Rokka et al. [20], which showed a clear relationship between the microbiological quality of poultry (protein-based) and the total amount of biogenic amines. In addition, Mattila et al. [21] found a correlation between CO_2 concentration and the growth of microorganisms in pea and tomato soups which were packaged aseptically either in air or in a mixture of oxygen (5%) and nitrogen.

Table 2
Changes in CO_2 and microbial counts of golden drop during storage at 25 °C.

Time (days)	Mesophilic bacteria[a] (log CFU g^{-1})	Yeast and mold (log CFU g^{-1})	Osmophilic yeast (log CFU g^{-1})	CO_2 (%, v/v)
0	2.53 ± 0.07	1.22 ± 0.08	2.56 ± 0.07	0.000 ± 0.001
0.5	2.73 ± 0.08	1.52 ± 0.08	2.78 ± 0.07	0.062 ± 0.003
1	2.81 ± 0.08	1.92 ± 0.08	2.81 ± 0.07	0.085 ± 0.003
1.5	2.85 ± 0.08	2.12 ± 0.08	2.83 ± 0.08	0.110 ± 0.005
2	2.91 ± 0.08	2.22 ± 0.08	2.92 ± 0.07	0.127 ± 0.004
2.5	3.07 ± 0.08	2.30 ± 0.08	2.94 ± 0.07	0.145 ± 0.006
3	3.28 ± 0.08	2.34 ± 0.08	3.03 ± 0.07	0.163 ± 0.006
3.5	3.29 ± 0.08	2.50 ± 0.08	3.07 ± 0.08	0.181 ± 0.006
4	3.54 ± 0.09	2.59 ± 0.08	3.12 ± 0.08	0.219 ± 0.005
4.5	3.67 ± 0.08	2.71 ± 0.08	3.19 ± 0.08	0.231 ± 0.004
5	3.72 ± 0.09	2.77 ± 0.08	3.56 ± 0.07	0.261 ± 0.007
5.5	3.96 ± 0.08	3.11 ± 0.08	4.04 ± 0.07	0.312 ± 0.007
6	4.27 ± 0.07	3.31 ± 0.08	4.26 ± 0.08	0.403 ± 0.005
6.5	4.31 ± 0.10	4.14 ± 0.08	4.38 ± 0.07	0.610 ± 0.004
7	4.45 ± 0.10	4.30 ± 0.08	4.65 ± 0.07	0.672 ± 0.004
7.5	4.73 ± 0.09	4.59 ± 0.08	4.72 ± 0.08	0.958 ± 0.007
8	5.02 ± 0.09	4.71 ± 0.08	4.75 ± 0.08	1.238 ± 0.006
8.5	5.30 ± 0.09	5.09 ± 0.08	4.92 ± 0.08	1.888 ± 0.005
9	6.01 ± 0.09	5.45 ± 0.07	5.74 ± 0.08	2.939 ± 0.004

[a] Mean ± standard deviation of triplicate determinations of two batches.

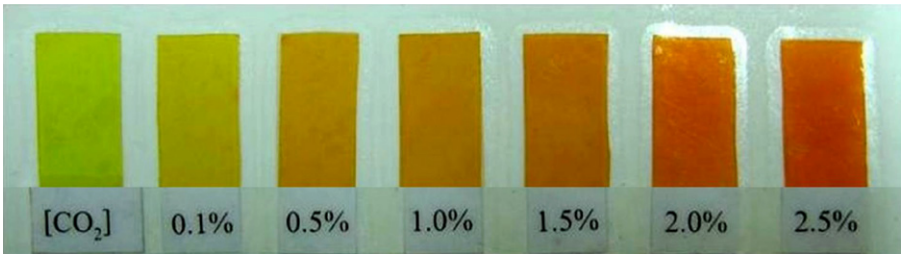

Fig. 3. Change in color of indicator label in response to CO_2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.2. Color changes of indicators

When CO_2 was added to vials containing colorimetric mixed-dye-based indicator solution, a visual color change of the solution from bright light green to bright red 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 430–526 nm (Fig. 2). 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 615–618 to 430–435 nm. Methyl red, which shifts from basic form (yellow, pH 6.2) to acidic form (red, pH 4.5), results in maximum lambda (λ_{max}) shifting from 430–435 to 523–526 nm [22]. As a mixed-dye-based indicator, bright light green changed to bright red in relation to a shift in the absorption peak from 430–435 to 523–526 nm when exposed to levels of CO_2 from 0% (v/v) to 3.5% (v/v). This vivid color spectrum of mixed-dye indicator solution is in agreement with Wallach [23], who reported that a mixed indicator could enhance an expansion of the range of color change, as compared with a single indicator.

The visual color changes of colorimetric mixed-dye-based indicator labels are depicted in Fig. 3. It was found that indicator labels showed a clear spectrum from bright light green to orange-red when exposed to a level of CO_2 in a range of 0–2.5% (v/v). The main purpose for 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 to the TCD values of each indicator label.

3.3. Color changes of indicator labels during golden drop spoilage trial

Fig. 4 shows the change in CO_2 level monitored by colorimetric mixed-dye-based indicator labels in golden drop at 25 and 10 °C. These findings showed that the minimal spoilage level of golden drop occurred on days 6 and 28 during storage at 25 and 10 °C, respectively. Indicator labels showed a clear spectrum from bright light green to orange-red when exposed to CO_2 during storage time. Carbon dioxide from headspace dissolved in the filter layer, which is hydrophilic material, forms carbonic acid in the presence of moisture. Carbonic acid is diprotic, having two hydrogen atoms which may dissociate from the parent molecule, forming hydrogen ions (H^+) and bicarbonate ions (HCO^{3-}) with pK_a of 6.36 at 25 °C [24]. Then, as a proton, a hydrogen ion combines with 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

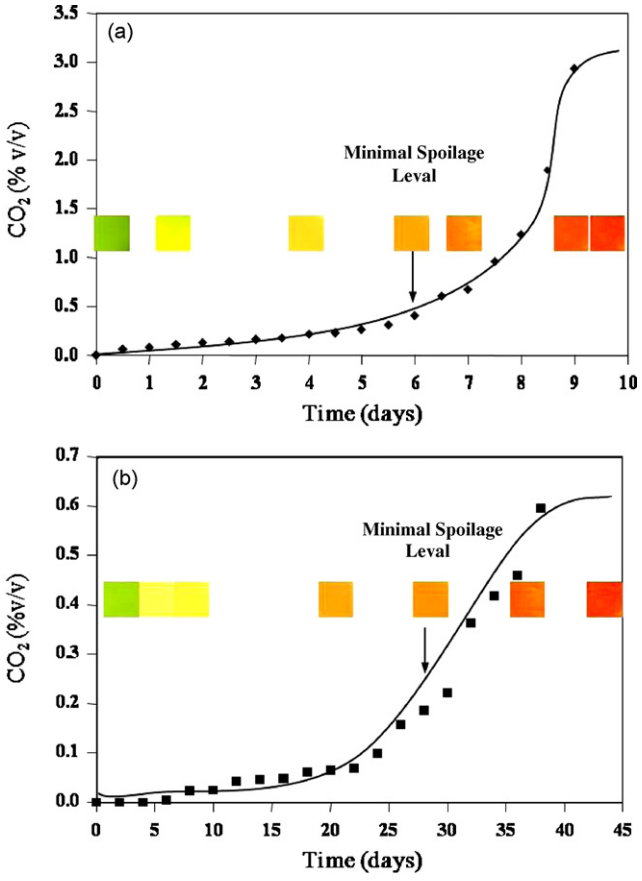

Fig. 4. Change in CO_2 level in golden drop at 25 °C (a) and 10 °C (b), with indicator label which show color change over time. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

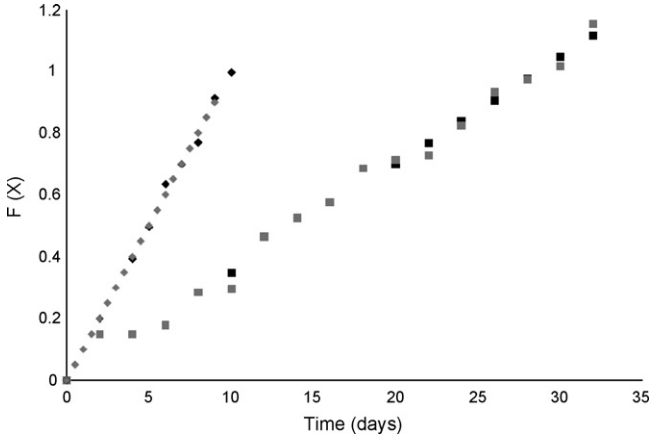

Fig. 5. Plot of response function $F(X)$ with time for CO_2 level in golden drop (■) 10 °C, (◆) 25 °C; and for total color difference in indicator labels (■) 10 °C, (♦) 25 °C.

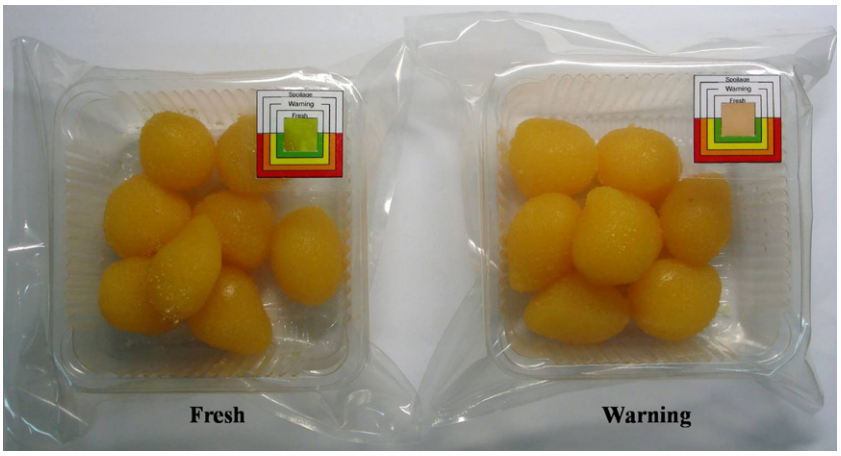

Fig. 6. Packaged golden drop with food spoilage indicator label. Green = fresh; orange = warning. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

[22]. Golden drop is highly nutritious (protein from egg yolk, carbohydrate from sugar and rice flour). It possesses a slightly acid pH (6.60) and high moisture content (25–28%), and 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 [12].

The changes of CO_2 as a result of golden drop spoilage can be modeled theoretically in accordance with Hong and Park [25]. The CO_2 level of golden drop consistently changed over time, represented by a sigmoidal curve. Polynomial functions give the lines of this curve, represented by the following equation:

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

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 10 °C, the function is appended below:

$$X = 0.7011x^6 - 5.0768x^5 + 11.757x^4 - 10.804x^3 + 4.1955x^2 - 0.4228x$$

At 25 °C, the function is appended below:

$$X = 47.909x^6 - 107.31x^5 + 90.588x^4 - 33.648x^3 + 4.8768x^2 + 0.0502x$$

The above equation can be rearranged to the desirable form:

$$F(X) = \left[\ln\left\{\frac{1}{1-X}\right\}\right]^{1/n} = kt$$

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. 5). The rate constants and the coefficients of determination for CO_2 levels of golden drop at different temperatures are given in Table 3. Based on Arrhenius equation, carbon dioxide formation during golden drop spoilage yields an activation energy $E_a = 48.98\ kJ\ mol^{-1}$ in comparison with the organic production during kimchi fermentation which had $E_a = 76.8\ kJ\ mol^{-1}$ [25]. Activation energy is an empirical parameter characterizing the exponential temperature dependence of the rate constant. In particular, the activation energy indicates the temperature sensitivity of the spoilage response.

Table 3
Response rate constants and coefficients of determination[a] for CO_2 of golden drop and for total color difference (TCD) of indicator label at different temperatures.

Temperature (°C)	CO_2		TCD	
	k (day^{-1})	r^2	k (day^{-1})	r^2
10	0.0351	0.9927	0.0349	0.9782
25	0.1001	0.9905	0.1001	0.9973

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

The TCD value also changed continuously and consistently with the response of the indicator label. TCD values gradually increased with time. The final TCD values (coating side) were 26.32–30.18. It is generally known that TCD values greater than 5.0 can be easily detected by the unaided eye, and TCD greater than 12.0 indicates a completely different color space [26]. 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. 5), if $F(X) = kt$ is defined as the response function. The rate constants and coefficients of determination for TCD of indicator labels at different temperatures are given in Table 3. Total color difference of indicator labels resulted in E_a of 49.25 kJ mol^{-1}. If the food spoilage indicator and the food product quality deterioration reactions have similar temperature dependence, translated into activation energy differing by less than 5 kJ mol^{-1}, the indicator can be accurately used to monitor the freshness of food products. Packaged golden drop with food spoilage indicator label is shown in Fig. 6.

4. Conclusions

The results presented in this study indicated that fast and sensitive detection of spoilage metabolites in intermediate-moisture Thai dessert can be achieved by a non-invasive colorimetric method. The indicator response was found to correlate with microbial growth patterns in golden drop samples, therefore enabling the 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 certainly of primary interest to consumers. In addition, product confidence is of great importance to manufacturers and retailers in order to protect their brand value from customer dissatisfaction. The expansion of the concept of a colorimetric mixed-dye-based food spoilage indicator

to other food products – such as minimally processed foods, easily prepared foods, fermented foods, fresh meat and meat products, poultry and poultry products, seafood and seafood products, bakery and bakery products, desserts, and fresh-cut fruits and vegetables – is a possible area for future research.

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