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

Chompoonoot Rukchon [a], Atchareeya Nopwinyuwong [a,c], Sudsai Trevanich [b,c], Tunyarut Jinkarn [a,c], Panuwat Suppakul [a,c,*]

[a] Department of Packaging and Materials Technology, Faculty of Agro-Industry, Kasetsart University, 50 Ngamwongwan Rd., Chatuchak, Bangkok 10900, Thailand
[b] Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, 50 Ngamwongwan Rd., Chatuchak, Bangkok 10900, Thailand
[c] Center for Advanced Studies in Agriculture and Food, KU Institute for Advanced Studies, Kasetsart University, 50 Ngamwongwan Rd., Chatuchak, Bangkok 10900, Thailand

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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

* Corresponding author at: Department of Packaging and Materials Technology, Faculty of Agro-Industry, Kasetsart University, 50 Ngamwongwan Rd., Chatuchak, Bangkok 10900, Thailand. Tel.: +66 2 562 5058; fax: +66 2 562 5046.
E-mail address: fagipas@ku.ac.th (P. Suppakul).

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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.