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Regression analysis for predicting the fermentation state of packaged Kimchi using a colorimetric indicator

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ABSTRACT

The application of a colorimetric indicator for monitoring the degree of fermentation in Kimchi was studied. Changes in total color difference (TCD) occurred continuously from the initial to final fermentation stage with a maximum TCD value of 36.08 ± 0.72 after 28 days. The experimental data for the converted color response function, *F(Xc)* were demonstrated to be more linear ($R^2 = 0.986$) than for TCD values. The coefficients of determination of pH: *F(Xc)* ($R^2 = 0.9583$) and titratable acidity: *F(Xc)* ($R^2 = 0.9828$) were sufficient to meet the zero-order reaction. Results of standardized residuals evidenced their normal distribution, indicating that 95% of the residuals of predicted pH or titratable acidity were in the range of -1.96 to 1.96. The coefficients of determination between predicted- and observed data were 0.841 (pH) and 0.912 (titratable acidity). Based on regression analysis, the colorimetric indicator could thus be applied as a Kimchi fermentation indicator.

1. Introduction

Kimchi, listed in the Codex Alimentarius in 2001 (CODEX STAN 223-2001), constitutes a major component of Korean food as a traditional salad of fermented vegetables comprised of cabbage pickled in salt and mixed with spices and various seasonings (Lee et al., 2017). Cabbage Kimchi, utilized worldwide as a fermented vegetable with various health benefits, is considered a vegetable probiotic food that enhances health (Lee et al., 2011). According to Mheen and Kwon (1984), Kimchi exhibits its most attractive taste, flavor, and texture when properly fermented at a pH of approximately 4.2 and 0.6–0.8% of titratable acidity. After optimum fermentation, the sensory quality of Kimchi declines gradually owing to the formation of noticeable organic acids such as lactic and acetic acids. Moreover, the quality of commercial Kimchi obtained by consumers in the market may vary with respect to degree of fermentation, depending on temperature, and the time between packaging and consumption, as lactic acid fermentation continues to occur in packaged kimchi until the expiry date is reached. Therefore, the application of new smart packaging devices such as colorimetric indicators, sensors, or labels that are applied to the package has been routinely attempted in the Kimchi industry to indicate the degree of product fermentation and freshness.

Colorimetric indicators attached to the inside of a package to observe the alteration in the properties of packaged product can facilitate consumer decision making by confirming food quality without requiring tasting of the product. Colorimetric indicators are generally utilized as an observable response of color factors that correlates to the condition of a food product at a certain temperature (Taoukis, 2008). However, although various new types of colorimetric indicators have recently been developed and are commercially available in several countries (Riva et al., 2001; Wanihsuksombat et al., 2010; Yan et al., 2008), there remains a lack of study regarding the application of colorimetric indicators with respect to monitoring Kimchi fermentation stage.

From the early stages of Kimchi fermentation, typical lactic acid bacteria such as *Lactobacillus plantarum, Lactobacillus brevis* and *Leuconostoc mesenteroides* gradually increase under microaerobic growth conditions to consistently generate lactic acid, acetic acid, volatile organic compounds (VOCs), ethanol, and CO_2 gas (Cho et al., 2006; Mheen and Kwon, 1984). Therefore, the concentration of fermentation by-products (FBPs) within the packaged Kimchi rises significantly, with the changes in FBP concentration showing positive association with the titratable acidity and pH of fermented Kimchi (Hong et al., 1994, 1995; 1996a, 1996b; Hong and Park, 1997). Acidic substances or VOCs such as acetic acid that diffuse into the receptor of a colorimetric indicator induce a gradual color change (Mendoza et al.,

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Abbreviations: FBP, fermentation by-product; TCD, total color difference; VOC, volatile organic compound

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2004; Shimoni et al., 2001). Color changes in chromogenic devices under the background of variance in FBP concentrationare defined using color values such as the color response value (Giannakourou and Taoukis, 2002; Giannakourou et al., 2005; Lee and Lee, 2008), light reflectance (Bobelyn et al., 2006), lightness (Shimoni et al., 2001), the yellow/blue opponent co-ordinate (Nuin et al., 2008), and total color difference (Tsironi et al., 2008; Vaikousi et al., 2009; Wanihsuksombat et al., 2010) to define each colorimetric indicator color (Mendoza et al., 2004).

With respect to the use of colorimetric indicators for monitoring fermentation in Kimchi, some colorimetric indicators for FBP detection have been developed and evaluated regarding their applicability to Kimchi packaging based on emissions of VOCs during the fermenting stage, which can be used as a chemical index for the fermentation in fermented food (Feng et al., 2010a, 2010b; Hong and Park, 2000; Lim et al., 2009; Lin and Suslick, 2010; Lin et al., 2011). However, although color changes of the indicators have verified their suitability for consumer decision making, a reliable correlation between color factor and Kimchi FBP indicators remains unclear because previous studies used a self-produced indicator prototype rather than commercially available products. The aim of the present study was therefore to develop a primary regression model based on the correlation between the response of a commercially available colorimetric indicator and titratable acidity or pH to estimate the fermentation stage of Kimchi during storage at 4 °C, 10 °C and under fluctuating temperature conditions.

2. Materials and methods

2.1. Preparation of Kimchi samples and the colorimetric indicator

Commercial Kimchi was purchased from a local market in Gwangju, Korea. Each Kimchi sample was packed individually in 2 kg units and stored in a rectangular Kimchi container (LOCK&LOCK 8L, Seoul, Korea) at 4 °C and 10 °C for 28 days. Additional samples were exposed to fluctuating temperatures by incubation either in a temperature-controlled chamber or in a refrigerator with a programmable temperature history function. Each sample had six replicates. Following incubation at a range of temperatures fluctuating from 0 to 18 °C (simulation 1: 0–4 °C, simulation 2: 0–10 °C, and simulation 3: 0–18 °C) a random sampling method was used to collect samples for this study. The fermentation state of packaged Kimchi under fluctuating temperature condition was monitored using real time temperature logging technology (Signatrol SL52T, Signatrol Ltd., Tewkesbury, UK), which included Radio Frequency Identification. A kimchi fermentation stage sensor (SENKO Co., Ltd., Gyeonggi-do, Korea), a commercially available colorimetric diffusion-based indicator for monitoring Kimchi fermentation stage that relies on the color development of a chemical system reaction upon VOC-dependent permeation into a film, was attached to the inner side surface of the container. According to the manufacturer's instructions, a kimchi fermentation stage sensor is a colorimetric indicator composed of a dye mixture solution of various compounds such as polyethylene glycol, ethanol, bromothymol blue, and chlorophenol red that exhibits gradual colour changes in response to an acidic substance or VOC value to indicate the acidity level. The membrane is composed of hydrophilic cellulose fiber. An indicator solution with a concentration of 1% (v/v) in ethanol (50%, v/v) was produced by mixing chlorophenol red (0.1%, w/v) in ethanol (50%, v/v) and bromothymol blue (0.1%, w/v) in ethanol (50%, v/v) in a ratio of 2:3. All experimental results are presented as the average and standard deviation of multiple measurements ($n \geq 5$).

2.2. Measurement of organic acid content, pH and acidity

Each samples (5 g of cabbage and 5 g of Kimchi juice) was homogenized in a blender (Philips HR1372, Guildford, UK), 25 mL of distilled water was added to 1 g of sample, organic acids were extracted for 30 min using a sonicator (Power Sonic 520; Hwashin Tech Co., Daegu, Republic of Korea) and filtered twice, through a Toyo No. 1 and a syringe filter (RC, 0.2 μm, 25 mm). A 1260 Infinity/G4212B high performance liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) with a variable wavelength detector (DAD) set at 210 nm was used. The injection volume was 10 μL. Organic acids were analyzed using an Aminex HP-87H column (300 × 7.8 mm, 9 μm) (Bio-Rad, Hercules, CA, USA) kept at 50 °C. An isocratic elution was performed with 0.008 M sulfuric acid in deionized water as mobile phase for 30 min (flow rate 0.6 mL/min). Organic acids in the samples were identified by comparing their retention times with those of lactic acid and acetic acid (Sigma, St. Louis, MO, USA), which were used as standards. They were quantified using a calibration curve derived from the peak areas of the standards.

Each Kimchi sample (5 g of cabbage and 5 g of Kimchi juice) was blended (Philips HR1372, Guildford, UK) and filtered using filter paper (8 μm, Whatman No. 2, Kent, UK). The pH and titratable acidity of Kimchi were determined using a TitroLine 5000 (SI Analytics, Mainz, Germany). The titratable acidity of each sample was measured following the standard Association of Official Analytical Chemists protocol. The Kimchi was titrated to pH 8.3 by addition of 0.1 N sodium hydroxide (NaOH; Daejung Chemical, Shiheung, Korea) solution, and the titratable acidity was calculated as the percentage of lactic acid by using the following formula (Eq. (1)):

$$\text{Titratable acidity (\%)} = \frac{0.1\ \text{N NaOH (mL)} \times 0.1\ \text{N NaOH factor} \times 0.009}{\text{Sample weight (g)}} \times 100 \tag{1}$$

2.3. Color measurements and kinetic parameters of the colorimetric indicator

The color changes of the colorimetric indicator were measured using a chroma meter (Konica Minolta CR-400, Osaka, Japan) and expressed as Hunter system (L^*, a^*, and b^*) values and the index of total color difference (TCD). The TCD defined by the National Bureau of Standards was used to compare the magnitude of total color changes throughout the storage period (Dowlati et al., 2013). The TCD value (ΔE) was calculated using the following equation (Eq. (2)) (Hong and Park, 2000).

$$\text{TCD value } (\Delta E) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \tag{2}$$

where color ΔL^*, Δa^*, and Δb^* are lightness, redness-greenness, and yellowness-blueness differences between color parameters determined on day 0 and color parameters determined after the indicated storage day, respectively.

Based on L^*, a^* and b^* values, an index was defined to quantify the total color difference of the colorimetric indicator using the chroma value equation (Eq. (3)) (Giannakourou and Taoukis, 2002) as follows:

$$C = \sqrt{(a^*)^2 + (b^*)^2} \tag{3}$$

According to Giannakourou and Taoukis (2002), the normalized chroma value (Xc) was used as the response X of the colorimetric indicator, which, when plotted as a function of time, had a sigmoidal shape, somewhat similar to a Gaussian function (expressed as $X = 1 - \exp[-(kt)^2]$). The normalized chroma value equation (Eq. (4)) was expressed as follows:

$$X_C = \frac{(C - C_{min})}{(C_{max} - C_{min})} \tag{4}$$

Accordingly, color response values of the colorimetric indicator can be expressed using the following form of a linearized response equation (Eq. (5)) as follows: