Chitosan-based carbon dioxide indicator to communicate the onset of kimchi ripening

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A B S T R A C T

The objectives of this study are to develop a chitosan-based CO₂ indicator that can notify the onset of ripening stage of packaged kimchi, and to study the correlation between the ripening stages of kimchi and the transparency levels of the chitosan-based CO₂ indicator during storage. The chitosan-based CO₂ indicators were developed by adding chitosan (0.4 g) and 2-amino-2-methyl 1-propanol (AMP, 5 mL) to distilled water (100 mL) at pH 7.0, subsequently packed in LDPE film sachets and placed in the headspace of kimchi packages. When the kimchi packages were stored for 2 weeks, the pHs of the chitosan-based CO₂ indicators decreased up to pH 5.8 at 10 °C and up to pH 5.9 at 20 °C. A remarkable change was observed in the degree of transparency of the indicators, i.e. from below 30% to nearly 100% at the end of the testing period. Onset point of optimal kimchi ripening (pH 4.2–4.4 and 0.4–0.8% of titratable acidity) was observed after 1 day storage at 20 °C and 5 day storage at 10 °C. The chitosan-based CO₂ indicator system offer a great potential as a convenient, easy-to-use technique for recognition of unripen packaged kimchi and/or onset of optimal ripening period.

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1. Introduction

Kimchi is a fermented food that is made by natural fermentation of vegetables; the fermentation process progresses even during storage and distribution after packaging of kimchi due to kimchi-inhabiting microbes. Therefore, kimchi usually has a limited shelf-life and quick sale is the top priority for kimchi manufacturers. Considering the post-packaging fermentation, freshly-made kimchi is packaged in its unripen state and on the other hand consumers tend to consume immediately or store under refrigeration after purchase. Therefore, there is a void of information about ripening stage between a manufacturer and a consumer. Fermentation of kimchi is occurred by various microorganisms and enzymes associated with ingredients/raw materials, and produce their unique fresh sour flavor by generating various organic acids. Therefore, during fermentation, pH and acidity of kimchi are the major indicators of product quality (Hong, Park, & Park, 1995). According to previous studies, the optimum ripeness of kimchi can be determined using the pH and acidity measurements. The optimum ripeness of kimchi was observed at the pH range of 4.2–4.4 and acidity around 0.6–0.8% (Lee, Cho, & Pyun, 1991). In case of insufficient fermentation, kimchi may exhibit high pH and low acidity. On the other hand, kimchi can tastes sourer due to low pH and high acidity when over-fermented. Therefore, consumers demand for real-time information on product quality or optimal ripeness is on high. However, at present, there’s no simple technique available that can be used to determine the optimal ripeness. The fermentation of kimchi is regulated by temperature and salinity, and it is difficult to determine degree of ripeness without the assistance of sensor system since kimchi is packed soon after preparation or shipped after low-temperature ripening when mass-produced (Hong & Park, 1997). Therefore, the development of detection technology that can be for determining visually the degree of ripening of kimchi in real-time is desperately needed.

In packaged kimchi, partial pressure of carbon dioxide is one important factor that indicates product quality since lactic acid bacteria in kimchi continually produce carbon dioxide as a byproduct of fermentation during storage and distribution process. Under packaged conditions, partial pressure profile of carbon dioxide changes with storage temperature and time, and an indicator representing changes in carbon dioxide levels in package headspace can provide information about the quality of kimchi indirectly. Chitosan is produced commercially by deacetylation of chitin, and is abundant natural polymer, and is composed of (1→4)-2-amino-2-deoxy-β-D-glucan (Xu, McCarthy, Gross, & Kaplan, 1996). When chitosan dispersed in neutral solvent like water, the solution becomes murky because of semi-crystalline nature of chitosan in...
the solid state (Rinaudo, 2006). On the other hand, chitosan can easily form acidic conditions due to increased degree of protonation (Rinaudo, Pavlov, & Desbrieres, 1999). This property of change in solubility of chitosan upon pH change can be adopted to develop a visual indicator system. The working principle is very simple that the accumulated carbon dioxide inside packaged kimchi which is produced due to fermentation decreases pH of solution containing chitosan, thereby turbid solution becomes transparent due to chitosan dissolution under decreased pH conditions. Carbon dioxide is readily soluble in water (1449 mg/L), and dissolved carbon dioxide reduces the pH of any water-based solution by forming carbonic acid (Johnson, 1982). For this reason, chitosan dispersion that functions in binary manner (on-off type) from opacity to transparency can be used to detect degree of ripeness of kimchi. Recently, a proof-of-concept study has been performed to examine the potential of chitosan-based carbon dioxide indicator (Jung, Puligundla, & Ko, 2012).

Carbon dioxide produced in packaged kimchi can be readily detected using chitosan suspension (indicator) which exhibits transparency change upon CO2 dissolution. Carbon dioxide is readily soluble in water; but if the rate of dissolution is controlled, and that property can potentially be used to design an indicator (Puligundla, Jung, & Ko, 2012). In particular, a method that addresses consumer requirement of visual indication of ripening degree of kimchi is necessary for practical use in packaged kimchi. In this study, a technique that control solubility of carbon dioxide was applied to chitosan-based carbon dioxide indicator. 2-amino 2-methyl 1-propanol (AMP) is a kind of alkanolamine (Xiao, Li, & Li, 2000), and it can accumulates carbon dioxide by forming carbonate due to preferential reaction with carbon dioxide (Sartori & Savage, 1983), and has been reported to react with a high rate (Yih & Shen, 1988). In this study, the visual difference of indicator was strengthened as carbon dioxide absorption of chitosan suspension has controlled and improvement in difference of transparency was achieved by adding AMP to chitosan-based dispersion.

The purpose of this study is to check the applicability of visual indicator in kimchi package which is developed using chitosan-AMP indicator solution and to study the correlation between the indicator transparency changes and degree of kimchi ripeness under defined storage temperatures and periods.

2. Materials and methods

2.1. Materials

Chitosan powder (Mw 22 kDa, deacetylation degree 84%) was provided by Kittolife Co., Ltd. (Pyeongtaek, Korea). 2-amino 2-methyl 1-propanol (AMP) was purchased from Acros Organics (Geel, Belgium). Hydrogen chloride and sodium hydroxide were purchased locally.

2.2. Preparation of chitosan-based indicator solution

In order to prepare a chitosan solution for detection of carbon dioxide, AMP in the range of 0–10 mL was mixed in 100 mL of distilled water and 0.4 g chitosan added. The pH of the chitosan solution was adjusted to 7 using dilute HCl and stirred at 700 rpm for 2 h. Final volume was made to 200 mL by adding distilled water and the pH of the chitosan solution was adjusted to 7.

2.3. Preparation of kimchi

Information on preparation procedure and ingredient ratio for kimchi was provided by the kimchi information service system (KISS). Briefly, after trimming, cabbage was cut into quarters widthwise and soaked in a brine solution which was prepared by adding bay salt (0.25 kg/kg cabbage) to 1.2 kg water, for 16 h at room temperature. Salted cabbage was washed twice, and drained off excess water for 1 h. Thereafter, cabbage pieces were mixed with other kimchi ingredients, seasoned, and finally packaged. The percentage composition of Kimchi ingredients is given in Table 1.

2.4. Kimchi packaging and indicator attachment

The chitosan-based indicator solution (5 mL each) for carbon dioxide detection was sealed into a square shaped (5 cm × 5 cm) plastic sachets which are made of low density polyethylene (LDPE) film with a thickness of 50 μm (CO2 permeance 175 mL m⁻² h⁻¹ atm⁻¹). The kimchi package (22 cm × 15 cm) is made of nylon/polyethylene/linear low-density polyethylene (Nylon/PE/LLDPE) film with a thickness of 110 μm (CO2 permeance <10 mL m⁻² h⁻¹ atm⁻¹) and almost impermeable to gases. The indicator sachet bag was placed in the headspace of kimchi package containing 100 g of kimchi at a sufficient distance avoiding direct contact, and it was kept intact by heat sealing process as depicted in Fig. 1. Properly sealed kimchi packages were stored at 10 ± 2 or 20 ± 2 °C in a low temperature cabinet until further analysis.

2.5. pH and titratable acidity of kimchi

Pulp suspension obtained after grinding of packaged kimchi (100 g for 1 min) was filtered through two pieces of gauze, and pH of the filtrate was measured. Titratable acidity was measured by using 10 mL kimchi filtrate. The sample titrated against 0.1 N NaOH until pH 8.3 was reached and measurements repeated 3 times; and finally titratable acidity values were calculated by using the following formula:

\[ \text{Titratable acidity} \times 100 = \frac{0.1 \text{N NaOH} \times 0.1 \text{N NaOH factor} \times 0.009}{\text{Sample} \times 100} \]

2.6. Enumeration of lactic acid bacteria

Kimchi filtrate samples were diluted by 10 times using sterile saline. After pouring and spreading 1 mL of each diluted sample onto the MRS agar medium (Difco Laboratories, Detroit, MI, USA), the plates were incubated at 43 °C for 48 h, colonies grown on the agar plate were counted to determine CFU (colony forming units)/mL. MRS agar medium was prepared from 55 g MRS broth, 15 g agar

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brined cabbage</td>
<td>100</td>
</tr>
<tr>
<td>Green onion</td>
<td>3.1</td>
</tr>
<tr>
<td>Dried red pepper powder</td>
<td>2.3</td>
</tr>
<tr>
<td>Garlic</td>
<td>1.5</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
</tr>
<tr>
<td>Ginger</td>
<td>0.4</td>
</tr>
</tbody>
</table>
(Difco Laboratories, Detroit, MI, USA), and 0.01 g BPB (Bromophenol blue, Sigma–Aldrich, St. Louis, MO, USA) in 1 L distilled water.

2.7. Signal response determination of chitosan-based CO2 indicators during the kimchi fermentation

The pH of CO2 indicator in the kimchi packages was measured during the kimchi fermentation at 10 and 20 °C for 2 weeks. The pH-dependent change in transparency of the CO2 indicator was measured at 600 nm wavelength using a UV-spectrophotometer.

2.8. Correlation study between the kimchi parameters and the CO2 indicator parameters

The CO2 indicator parameters were correlated to the kimchi parameters over time during the kimchi fermentation. The kimchi parameters were pH, titratable acidity and lactic acid bacteria while the indicator parameters were pH and transparency during the kimchi fermentation at 10 and 20 °C for 2 weeks. Microsoft’s Excel spreadsheet program was used to analyze the level of correlation between the kimchi parameters and the CO2 indicator parameters through its correlation function.

3. Results and discussion

3.1. Growth profile of lactic acid bacteria in kimchi during the storage

The growth profile of lactic acid bacteria in kimchi stored at 20 °C is shown in Fig. 2(A). The viable counts of lactic acid bacteria were about 10^5 CFU/mL immediately after packaging of kimchi. However, after 24 h storage, the count of lactic acid bacteria has increased rapidly to greater than 10^8 CFU/mL. So, it clearly indicates that the fermentation progressed rapidly soon after packaging. The total viable counts of lactic acid bacteria were stable throughout storage period after initial rise.

Lactic acid bacteria counts in packaged kimchi stored at 10 °C are shown in Fig. 2(B). Immediately after preparation, the number of lactic bacteria was 10^3 CFU/mL and the count began to increase slowly since kimchi was stored at suboptimal temperature of 10 °C. By the end of 4 days of storage, the total viable count was increased to 10^8 CFU/mL. The viable count decreased slightly during further storage however the change observed was insignificant.

The final total bacteria count level reached by the end of storage period was independent of initial count at both the temperatures. It is assumed that fermentation processes are affected by raw materials, temperature and so on. Lactobacillus growth rate of kimchi which stored at 10 °C was slower, but the final counts of lactic acid bacteria per unit mass of kimchi were similar to that of kimchi fermented at 20 °C. Therefore, it can be concluded that storage temperature can affect the growth rate of lactic acid bacteria.
3.2. Titratable acidity and pH of kimchi

The pH changes in kimchi stored at 20 °C are shown in Fig. 3(A). The pH of kimchi was 5.9 immediately after preparation, a significant decrease was observed thereafter. Active fermentation of kimchi was observed in the beginning of storage, and pH sharply decreased to 4.0 within four days. Further decrease was slow, and final pH of kimchi had reduced to 3.7 after two weeks of storage. The changes in pH of kimchi stored at 10 °C are shown in Fig. 3(B). The initial pH was 5.9 and steadily declined thereafter. After 9 days of storage, pH was reduced to 4. Compared with kimchi stored at 20 °C, the final pH of kimchi at 10 °C was slightly high. So, kimchi stored at higher (at 20 °C) temperature was more acidic in nature.

Since kimchi is a fermented food containing lactic acid, total acidity was calculated from the quantity of lactic acid produced as shown in Fig. 3(C and D). Titratable acidity profile changes depending on the progress of the fermentation. The initial titratable acidity of kimchi was about 0.2% but, steadily increased over 10 days at 20 °C, the rate of increase was slightly decreased thereafter. Final titratable acidity of kimchi was increased to about 2.0%. It is well known that kimchi is delicious to consume when its pH is around 4.0 and titratable acidity is 0.75%. Usually, kimchi stored at 20 °C reaches optimal ripening after 2 days' fermentation, whereas in the present study, pH of 4.2−4.4 and acidity of 0.4−0.8% were observed after 1 day of storage. This could, however, be due to enhanced ripening process which is based on other fermentation factors including initial count of bacteria.

Titratable acidity of kimchi stored at 10 °C was 0.3% initially, and then remained constant for 3 days; thereafter slowly increased until 9 days of storage and attained 1.0% titratable acidity. Since then titratable acidity was maintained constantly without significant change. Titratable acidity of kimchi stored at 20 °C was high, increased up to 2.0%, compared to the final acidity of kimchi stored at 10 °C (1.0%).

Like depending on the storage temperature the pH of kimchi differs, acidity of kimchi is also dependent on temperature favoring the proliferation of fermenting bacteria and frequency of new cells appearance (Shin, Kim, Han, Lim, & Park, 1996). Optimal ripening condition of pH 4.2 and titratable acidity 0.75% was reached in relatively short time, in 2 days, compared to previous reports (Hong, Park, & Park, 1994a, 1994b; Hong & Park, 1997).

3.3. Signal response of indicator during kimchi fermentation

The change in the pH of indicators during kimchi fermentation at 20 °C is shown in Fig. 4(A). The pHs of control indicators were around 7 with insignificant changes over time. However, indicator solution in kimchi package exhibited a rapid decrease of pH with the progress of fermentation process. It was observed that the pH of indicators in kimchi package was declined to 6 soon after storage. After 1 day of storage, the pH of indicators that are without and with AMP (5 mL of AMP in 100 mL of solution) were 5.9 and 5.8, respectively. Indicator with AMP exhibited lower pH than indicator without AMP. From these results, it can be concluded that absorption of CO2 can be improved by adding AMP.

The initial transparencies of indicators without and with added AMP recorded were 27.0 and 13.3%, respectively. The transparency of indicator increased rapidly to more than 90% in indicator without AMP at 20 °C for 1 day. On the other hand, transparency of indicator with added AMP increased slightly and remained at 40% level thereafter. This result suggests that AMP causes the reaction

Fig. 3. Change in the pH at (A) 20 °C and (B) 10 °C and the titratable acidity at (C) 20 °C and (D) 10 °C of kimchis fermented for 14 days: CO2 indicator without AMP (■); CO2 indicator with AMP (●) in kimchi.
preferentially because unstable AMP in solution is higher reactivity
than carbon dioxide.

The pH change of indicators during the storage period at tem-
peratures of 20 and 10 °C are shown in Fig. 4. Control indicators
were placed inside the pouches without kimchi and a slight
decrease of pH from its initial value was observed during the
storage period. However, the difference was insignificant and didn’t
affect the transparency of indicator as shown Fig. 5. The indicator
that was attached inside kimchi containing package has exhibited a
rapid pH decline and a low pH was maintained throughout the
storage period. The final pH of indicator with AMP was 5.75 and
was lower than the final pH of indicator without AMP. At both
storage temperatures of 20 and 10 °C, the indicators with AMP
showed lower pH values and greater absorption of CO₂ than indi-
cators without AMP. Transparency profile of indicators over the
storage period is shown in Figs. 4 and 5; the indicators with AMP
exhibited a lower transparency. Whereas in the case of indicators
with added AMP, low (20%) transparency was observed in the
beginning, but started to rise over the storage time period, almost
100% transparency was noted by the end of tested period.

The usefulness of the CO₂ indicator parameters was validated
using the correlation study with the kimchi parameters. The levels
of correlation between the kimchi parameters and the CO₂
indicator parameters at 10 and 20 °C are listed in Table 2. The

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Correlation between the kimchi parameters (pH, titratable acidity and CFU of lactic acid bacteria) and the CO₂ indicator parameters (pH and transparency) fermented at 10 and 20 °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage temp.</td>
<td>Kimchi parameters</td>
</tr>
<tr>
<td>CO₂ indicator parameters</td>
<td>20 °C</td>
</tr>
<tr>
<td></td>
<td>10 °C</td>
</tr>
<tr>
<td></td>
<td>Transparency</td>
</tr>
</tbody>
</table>

The correlation between the kimchi parameters and the CO₂ indicator parameters was studied to examine how well the indicator ripened kimchi over storage time. The correlation between the pH and transparency of the CO₂ indicator and the CFU of lactic acid bacteria of kimchi was high indicating that the CO₂ indicator parameters are good indices to describe growth of lactic acid bacteria over time. In addition, the pH and transparency of the CO₂ indicator showed fair correlation with the pH and titratable acidity of kimchi during fermentation. Therefore, chitosan-based carbon dioxide indicator is suitable for use as quality indicator since it has good correlation with the pH and titratable acidity of packaged kimchi.
4. Conclusions

In this study, chitosan-based CO₂ indicators were developed and their applicability was tested for packaged kimchi. The final pH and titratable acidity of kimchi stored at 20 °C were changed to 3.7 and 2.0%, respectively; 4.0 and 1.0% was observed at 10 °C by lactic acid fermentation. Without the addition of AMP, indicators showed a great change by kimchi fermentation; its pH decreased to 5.9 and 5.8 at 20 and 10 °C fermentation temperature, respectively. In general, it is known that kimchi reaches optimum ripening at pH 4.2–4.4 and acidity 0.4–0.8% for 1 day and 5 days stored at 20 and 10 °C, respectively. The low transparency of the indicators was observed in the beginning, but started to rise over 1 day and 4 days stored at 20 and 10 °C, respectively. Therefore, the chitosan-based CO₂ indicators can indirectly represent the starting point of optimum ripening of kimchi since their parameters have good correlation with the quality parameters of packaged kimchi.

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References


