Control of Dongchimi Fermentation with Chitosan Deacetylated by Alkali Treatment to Prevent Over-Ripening

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Abstract: Antimicrobial activities of chitosan against lactic acid bacteria were studied to apply for controlling dongchimi (whole-radish juicy kimchi) fermentation to prevent over-ripening. Antimicrobial activity of chitosan against lactic acid bacteria such as Leuconostoc mesenteroides and Lactobacillus plantarum was assayed at 10, 20, 30, and 40 mg/L concentration in the medium. The addition of 40 mg/L of the chitosan prepared at 140 °C for 10 min showed strong inhibitory effect on the growth of L. mesenteroides and L. plantarum. The effects of addition of chitosan to dongchimi have also been studied during fermentation at different temperatures of 4, 10, and 20 °C. Addition of chitosan decreased markedly viable cell counts of lactic acid bacteria such as Leuconostoc spp. and Lactobacillus spp. at the initial stage. Subsequently the lactic acid bacteria recovered the growth to the same level as non-chitosan treated dongchimi. During the dongchimi fermentation, the addition of chitosan at larger quantity up to 1000 mg/L (CS1000) prolonged the palatable fermentation period. Addition of chitosan in the dongchimi seemed to inhibit the growth of lactic acid bacteria, thereby lowering the acid content. It, therefore, caused the shelf life to be extended and resulted in a prolonged palatable period for the dongchimi.

Keywords: antimicrobial activity, chitosan, dongchimi, kimchi, lactic acid bacteria

Introduction

In recent years, chitosan, a copolymer derived from the abundant natural polymer chitin (poly-β-(1,4)-N-acetyl-D-glucosamine), found in the shells of crabs and shrimp has been used in a wide variety of applications as biologically active substances in food and biomedical industries. Chitosan has been studied to possess several physicochemical and functional properties such as water and fat uptake (Cho and others 1998), emulsification (Knorr 1982), dye binding (Youn and others 2009), and antimicrobial activities (Sudharshan and others 1992; Zheng and Zhu 2003; Qin and others 2006). Chitosan have been used for various food applications including fruit juices, emulsified sauces, chilled salads, and mayonnaise (Roller and Covill 1999, 2000; Rhoades and Roller 2000; Oh and others 2001). The antimicrobial activity of chitosan is well documented; this is due to the interaction of NH\(^{+}\) groups on chitosan molecules with the negatively charged phosphoryl groups of phospholipid components on the microbial cell membranes (Liu and others 2004). Subsequently the growth of microorganisms is inhibited due to the unique biological and physicochemical activities of the positively charged chitosan molecules (Rabea and others 2003). For example, chitosan (0.1 g/L) reduced the viability of Lactobacillus spp. and Pediococcus spp. by 5 logarithmic cycles (Gil and others 2004). Thus, chitosan has attracted attention as a natural preservative for a variety of food applications (Roller and Covill 1999, 2000; Rhoades and Roller 2000; Tsai and others 2000).

Dongchimi is a Korean traditional juicy kimchi prepared with firm radishes, garlic, ginger, and pungent green peppers. Small and firm radishes are trimmed, cleaned, and stacked in a crock, and brine is poured over the vegetables along with the seasonings sufficient to cover them. Dongchimi is a liquid type fermented food having a cool and fresh sour taste of organic acids combined with adequate saltiness. Fermentation of dongchimi is usually carried out by a successive growth of lactic acid bacteria. Acids are continuously produced after the optimum ripening, which cause the softening of texture and produce undesirable taste and odor. These changes are called the over-ripening of dongchimi, which is the most serious problem in the fermentation of dongchimi. Hence, the best way to overcome the over-ripening is to control the growth of lactic acid forming microbes without affecting the quality of dongchimi. Several strategies such as irradiation, addition of salts, or addition of natural preservatives are favorable to inhibit the growth of lactic acid bacteria during dongchimi fermentation. The addition of natural preservatives, such as chitosan, is of interest because of its biocompatibility, nontoxicity, and antimicrobial action. Recently, application of chitosan is promising to improve the quality and extend shelf life of time-limited foods including fermented foods. Chitosan might be an ideal natural preservative for the dongchimi, but little literature is available for the application of chitosan in dongchimi.

The objectives of this study were: (1) to prepare the chitosans from chitin and to investigate effect of heating conditions on degree of deacetylation and antibacterial activities; and (2) to study the effects of addition of chitosan on the prolongation of palatable period of dongchimi by controlling fermentation, thus preventing over-ripening of dongchimi.
Control of dongchimi fermentation...

Materials and Methods

Purification of chitin

Crude chitin (commercial grade, 3.5% ash content) from the shell of a red crab (Chitonouesectes spioi) was obtained from Dongwoo Co. (Sockcho, Korea). In the 1st step, the chitin (1000 g) was dispersed in 20 L of 1 M aqueous HCl for 12 h under constant stirring. The chitin dispersion was filtered off to separate a solid phase. The acid-treated chitin filtrate was washed with deionized water and subsequently filtered again. In the 2nd step, the chitin filtrate prepared was dispersed in 20 L of 1 N aqueous NaOH at 100 °C for 1 h under stirring. The base-treated chitin was filtered and washed with deionized water until neutralization. The purified chitin prepared was centrifuged at 100 × g for 10 min and dried at 50 °C overnight. The amount of purified chitin was 950 g.

Preparation of chitosan

Chitosan was prepared by alkaline deacetylation of chitin (Wan and others 2003). The purified chitin (160 g) was dispersed in 4 L of 50% (w/v) aqueous NaOH for 30 min under stirring. The chitin dispersion was treated at three different heating temperature and time conditions, (1) at 100 °C for 6 h, (2) at 120 °C for 20 min, and (3) at 140 °C for 10 min. After washing 6 times with deionized water until neutralization, the chitosans prepared were centrifuged at 1000 × g for 10 min and dried at 50 °C overnight.

Measurement of degree of deacetylation for chitosan

Various physical and chemical properties of chitin and chitosan depend greatly on the degree of deacetylation. The degree of deacetylation is one of the most important factors for specifying the characteristics of chitin and chitosan. IR spectroscopy was to determine the degree of deacetylation for chitin based on the base line method with the absorbance ratio of A1550/A2878 (Sannan and others 1978). A chitin sample was dispersed in 1% (w/w) KBr solution, and subsequently formed a thin layer under certain pressure. The layered chitin was measured using an IR spectrophotometer (Magna-550, Nicolet Instrument Corp., Madison, Wis., U.S.A.). The layered chitin sample was measured at 500 to 4000 cm⁻¹ and compared with the degree of deacetylation from the absorption ratio of amide II band at 1550 cm⁻¹ to the CH stretching vibration at 2878 cm⁻¹ (Shimahara and Takiguchi 1988). The degree of deacetylation (%) was calculated using the absorption ratio A1550/A2878 in the equation of calibration curve. The determination range was 34.68–98.03.

Determination of molecular weight

The molecular weight of chitosan was measured by its viscosity. The molecular weight of chitosans is determined by measuring the viscosity using an Ubbelohde type viscometer and subsequent calculating the degree of polymerization in accordance with the Staudinger’s viscosity equation (Staudinger and Eder 1941) from the viscosity measured at a stable 25 °C.

Absolute viscosity ([η]):

\[ [\eta] = \lim_{C \to 0} \frac{\eta_C - 1}{C} \]

where \( C \), concentration.

Staudinger equation:

\[ [\eta] = K \cdot M_w^a \]

where \( K \), 8.93 × 10⁻⁴ cm²/mol·g⁻¹; \( M_w \), molecular weight; \( a \), 0.71.

Briefly, 0.4 g of chitosan was dissolved in 100 mL of an aqueous mixture of 0.2 M acetic acid, 0.1 M NaCl, and 4 M urea. The primary chitosan solution (0.4%) was diluted repeatedly with the same solvent of aqueous mixture of acetic acid, NaCl, and urea. The final concentrations of the chitosan mixtures were 0.4%, 0.2%, 0.1%, 0.05%, 0.025%, 0.0125%, and 0.00625%. The mixtures with 7 different chitosan contents were immersed at 25 °C in a water bath, and stored in a dark room for 10 d.

Determination of inhibitory effect of chitosan on lactic acid bacteria growth

Antimicrobial activity of chitosan against lactic acid bacteria such as Lactobacillus mesenteroides and Lactobacillus plantarum was assayed by colony count on incubated agar plates. The mixture of 100 mL of 1% (w/v) chitosan solution and 0.5 mL of acetic acid was autoclaved. The chitosan mixtures were then added to the MRSB (Difco Laboratories, Detroit, Mich., U.S.A.) media and inoculated with L. mesenteroides and L. plantarum, and incubated with shaking at 30 °C for 24 h. Final concentrations of chitosan were 10, 20, 30, and 40 mg/L. Initial number of the bacteria was 10⁵ CFU/mL. Every 4 h the medium of L. mesenteroides was spread out on the sodium azide-glucose agar plate while that of L. plantarum was on the sodium azide-glucose agar plate. The plates were incubated at 37 °C for 24 h, and the colonies were counted.

Preparation of dongchimi

Fresh radishes were washed and polished with hands. The washed radishes were horizontally cut into 4 pieces, and soaked into 3% NaCl of brine solution (1 : 1 w/v) in a jar. Other ingredients such as green onion (sliced), garlic (chopped), and ginger (chopped) were sliced or chopped. The weight ratios of green onion, garlic, and ginger to radish were 1%, 0.5%, and 0.2%, respectively. They were wrapped in fine cloth bag and placed at the bottom of the brine solution in the jar. To investigate the effect of chitosan on the over-ripening of the dongchimi, 0.15% (v/v) acetic acid and 0.05% (w/v) or 0.1% (w/v) chitosan (prepared at 140 °C for 10 min; Mw 336.5 kDa) were added into the brine solution. To investigate the antibacterial effect of chitosan concentration during dongchimi fermentation, 4 different groups were compared: no addition (CON), 300 mg/L acetic acid (AA300), 500 mg/L chitosan in the acetic acid (CS500), and 1000 mg/L chitosan in the acetic acid (CS1000). At this moment, all ingredients were in the jar together. Finally, the lid of the jar was covered and dongchimi samples were fermented at 4, 10, and 20 °C for 7 to 49 d.

Measurement of pH and titratable acidity of dongchimi juice

The pH of dongchimi juice was determined using a pH meter (Fisher Scientific Inc., Springfield, N.J., U.S.A.). The acidity of dongchimi juice was measured according to the AOAC method
Safety

respectively. Initial number of the bacteria was 105 CFU/mL. Deacetylation was saturated after 10 min. The molecular deacetylation increased within 10 min to reach 78% deacetylated at 140 °C. At 140 °C, the degree of deacetylation increased rapidly from onset to 20 min to obtain 78% deacetylated chitosan. At 140 °C, the degree of deacetylation increased within 10 min to reach 78% deacetylated chitosan. Deacetylation was saturated after 10 min. The molecular weights of the chitosans manufactured at 100 °C for 6 h, 120 °C for 20 min, and 140 °C for 10 min were 746, 852, and 336.5 kDa, respectively. At the heating condition of 140 °C for 10 min, the molecular weight of the chitosan was the smallest. These results are similar to the previous studies that molecular weight of chitosan decreased over time since chitin molecules were disintegrated into lower molecular polymers at high temperature (Bough and others 1978; No and others 2003; Tsaih and Chen 2003).

As chitosan is deacetylated from chitin, both the deacetylation reaction and the degradation reaction occur together since substituted groups on C(2) - C(3) positions of chitin molecules are in trans arrangement which retards the elimination of the acetyl group on the C(2) position, but not the degradation of β-1,4 linkage (Muna and others 1983; Tsaih and Chen 2003). Thus, deacetylation reactions are very effective at the early stage of heating, but become much ineffective during the later stage. The molecular weight also decreased rapidly in the 1st hour and slowly decreased afterward. Usually acetyl bond is fairly stable so that it is hard to break up the bond at low temperature. On the contrary, high temperature provides sufficient energy to detach the acetyl bonds from chitin structures. Therefore, high temperature heating should be employed for deacetylation to obtain lower molecular chitosan. From our results, the 140 °C condition produced chitosans with the smallest molecular weight (336.5 kDa). It might be concluded that the degradation in chitosan was the most severe at the highest temperature. Thus, heating temperature and time required to obtain 78% deacetylated chitosan were set to 140 °C and 10 min, respectively, in this study.

Growth inhibitory effect of chitosan on lactic acid bacteria L. mesenteroides. The main factors affecting the antibacterial activity of chitosans are molecular weight and concentration (Liu and others 2004). Antimicrobial activity of chitosan against L. mesenteroides in the medium is shown in Figure 1. For the chitosans prepared at 100 °C for 6 h and 120 °C for 20 min, 10 and 20 mg/L of the chitosan concentration were not effective on the growth inhibition against L. mesenteroides. In the case of 30 mg/L, the chitosan prepared at 120 °C for 20 min showed a minor inhibitory effect on the early stage of the bacterial growth. In contrast, its viability was considerably reduced at 40 mg/L of the chitosan concentration until 8 h incubation, but subsequently recovered as the growth level in the control group after 24 h incubation. Oh and others (2001) observed similar phenomena in the antimicrobial study against spoilage microorganisms in mayonnaise, and explained that the surviving cells may have been members of a more resistant subpopulation or may have survived because much of the chitosan was removed from the medium by binding to other cells.

For the chitosan prepared at 140 °C for 10 min, 10 and 20 mg/L of the chitosan concentration were not effective on the growth inhibition against the bacteria. The 30 mg/L of the chitosan prepared at 140 °C for 10 min significantly retarded the bacterial growth until 16 h incubation, but subsequently L. mesenteroides started growing. The final number of bacteria after 24 h incubation was 6.3 × 106 CFU/mL, which was less than 100th of the control group (approximately 107 CFU/mL). In conclusion, the 30 mg/L addition of the chitosan prepared at 140 °C for 10 min in the L. mesenteroides medium was effective on the inhibition of the bacterial growth. The addition of 40 mg/L of the chitosan prepared at 140 °C for 10 min showed strong inhibitory effect on the growth of L. mesenteroides. The viable cell count of L. mesenteroides was reduced from 105 CFU/mL up to less than 103 CFU/mL within 8 h incubation and thereafter, the bacteria were recovered to grow. After 24 h incubation, the number of L. mesenteroides only increased less than 100-fold (5 × 106 CFU/mL) from the initial number (105 CFU/mL) of bacteria. The 40 mg/L addition of the chitosan prepared at 140 °C for 10 min showed the strongest antibacterial effect in the L. mesenteroides medium. At lower chitosan concentrations (10 and 20 mg/L) there was no significant effect of molecular weight on the antibacterial activity. However, the antibacterial activity markedly increased with the smaller molecular weight chitosan (Mw 336.5 kDa) at higher concentrations of chitosan (30 and 40 mg/L). Similar results were observed when 0.1% chitosan with 224 kDa molecular weight was used to test its antibacterial activity against L. plantarum (No and others 2002).

L. plantarum. Growth inhibitory effect of the chitosans against L. plantarum is shown in Figure 2. For the chitosans prepared at 100 °C for 6 h and 120 °C for 20 min, the additions of the chitosan at 10, 20, and 30 mg/L did not show antibacterial effect against L. plantarum. However, chitosan at 40 mg/L concentration markedly inhibited growth of the bacteria, but the bacteria subsequently recovered as the growth level in the control group after 24 h incubation.

For the chitosan prepared at 140 °C for 10 min, the additions of the chitosans at 10 and 20 mg/L were also not effective on growth inhibition against the bacteria as in the case of L. mesenteroides. The 30 mg/L of the chitosan prepared at 140 °C for 10 min considerably inhibited the bacterial growth until 8 h incubation, but subsequently the bacteria proliferated up to the population of the control group (approximately 109 CFU/mL) after 24 h incubation. The addition of 40 mg/L of the chitosan prepared at 140 °C for 10 min inhibited significantly L. plantarum growth. However, the extent of inhibition was less severe than the case of L. mesenteroides. The viable cell count of L. plantarum was reduced from 105 CFU/mL up to 103 CFU/mL for 12 h incubation,
Control of dongchimi fermentation...

and subsequently remained less than the initial number (10⁵ CFU/mL) of bacteria throughout incubation. In conclusion, the 40 mg/L addition of the chitosan prepared at 140 °C for 10 min (Mw 336.5 kDa) showed the strongest inhibitory effect against the growth of \textit{L. plantarum}. Our results are similar to those of previous studies including the antimicrobial effect of chitosan on the growth of lactic acid bacteria. Chitosan at about 0.03% was effective on the growth inhibition of \textit{Lactobacillus bulgaricus}, \textit{Lactobacillus fermentum}, and \textit{Streptococcus faecalis} (Jeon and others 2001), and chitosan at 0.05% or more showed antimicrobial effect on the lactic acid bacteria growth (Sugawara and others 1997). We found that the 30 to 40 mg/L addition of the chitosan prepared at 140 °C for 10 min significantly inhibited the growth of both \textit{L. plantarum} and \textit{L. mesenteroides}. The effective inhibitory concentrations against the bacterial growth varied depending on the degree of deacetylation, molecular weights, and functional groups (Sudharshan and others 1992; Darmadji and Izumimoto 1994; Jeon and others 2001).

**Effect of chitosan on pH and acidity of dongchimi during fermentation**

Enzymes and microorganisms degrade components in radish and other ingredients during fermentation and concomitant products contribute to taste and flavor of dongchimi. More importantly, the fresh tastes of the dongchimi are mainly due to CO₂ and organic acids which are the derivatives from carbohydrates in the radish. Thus, acidity and pH are important quality indices of the kimchi family including dongchimi.

The pH of dongchimi juice was changed during fermentation. Figure 3(A), 3(B), and 3(C) are pH profiles of dongchimi juice fermented at 4 °C for 49 d, at 10 °C for 21 d, and at 20 °C for 7 d, respectively. Chitosan (prepared at 140 °C for 10 min; Mw 336.5 kDa)-added dongchimi juice obtained higher pH rather than CON. Addition of chitosan retarded pH decrease significantly during the dongchimi fermentation. The pH of AA300 was significantly low (approximately 4.5) as expected at the early stage of the fermentation compared to both CON and chitosan.
added groups (CS500 and CS1000). Overall, CS500 and CS1000 maintained higher pH values during the fermentation, but they decreased relatively fast up to the pH of CON at the last stage of the fermentation. Optimal pH for commercial dongchimi products is controlled at 4.2. At 4 °C fermentation, the optimal pH of CON was attained after 11 d, whereas that of CS500 was attained after 25 d. However, CS1000 did not reach up to pH 4.2 even after 49 d. At 10 °C fermentation, CON, CS500, and CS1000 reached pH 4.2 after 9, 17, and 18 d, respectively. The dongchimi juice quickly attained the optimal pH at 20 °C fermentation. CON, CS500, and CS1000 reached pH 4.2 after 2, 3, and 5 d, respectively.

Figure 3(A), 3(B), and 3(C) are the titratable acidity profiles at 4 °C for 49 d, at 10 °C for 21 d, and at 20 °C for 7 d, respectively, during dongchimi fermentation. Addition of chitosan delayed the acidity increase during the dongchimi fermentation. Acidity of the commercial dongchimi products is considered as optimal in the range from 0.13 to 0.21%. At 4 °C fermentation, the acidity of CON attained 0.13% after 18 d and increased up to 0.21% after 22 d, whereas that of CS500 was optimal after 28 d but exceeded 0.21% after 30 d. However, the acidity of CS1000 was favorable after 28 d, but did not exceed the highest limit after 49 d. At 10 °C fermentation, all groups including CON reached acidity 0.13% after 16 or 17 d. At 20 °C fermentation, CON, CS500, and CS1000 reached acidity 0.13% after 2, 3, and 4 d, respectively.

Addition of chitosan retarded pH drop and acidification in the dongchimi fermentation. The rate of both pH drop and acidity increase was suppressed by lowering fermentation temperature and/or adding chitosan which inhibited the growth of lactic acid bacteria and concomitant acid production. Retardation of pH decrease and acidity increase by chitosan addition has been reported in other kimchi researches. The fermentation time to reach to pH 4 to 4.2 was prolonged twice by addition of chitosan in cabbage kimchi (Seo and others 2004). The rate of the pH drop and the increase of acidity was delayed by addition of chitosan in radish kimchi (Kim and Kang 1994). In general, the addition of chitosan increased initial pH and subsequently retarded pH decrease during kimchi fermentation including dongchimi. In addition, chitosan provides a buffering effect in kimchi since NH₂ group takes H⁺ and concomitantly turns into NH₃⁺ in the acidic condition. Chitosan has a potential to provide a retardative effect on over-ripening during storage.

![Figure 2–Effect of chitosan on the growth of L. plantarum.](image-url)
Effect of chitosan on growth of lactic acid bacteria during dongchimi fermentation

In general, dongchimi with good quality means a well-fermented product whose acidity is in the range of 0.13% to 0.21%. As the acidity increases, the quality of dongchimi becomes worse (over-ripened). *Leuconostoc* spp, the major microorganism at the early stage of the dongchimi fermentation grows exponentially and attains to maximal population. During the growth, *Leuconostoc* spp. produces lactic acid and lowers pH in the dongchimi. Subsequently, *Lactobacillus* spp. replaces *Leuconostoc* spp. because the low pH inhibits the growth of *Leuconostoc* spp. but this is proper for the growth of *Lactobacillus* spp. (Park and others 2008). Among the three chitosans prepared under different conditions, the most effective chitosan (Mw, 336.5 kDa; degree of deacetylation, 78%) was chosen for the addition into dongchimi to prevent over-ripening by inhibiting the growth of *L. mesenteroides* and *L. plantarum*. 

*Leuconostoc* spp. Figure 4(A), 4(B), and 4(C) show the growth profiles of *Leuconostoc* spp. at 4 °C for 49 d, at 10 °C for 21 d, and at 20 °C for 7 d, respectively, during dongchimi fermentation. Population of *Leuconostoc* spp. in CON was higher than those in chitosan treated groups due to the antimicrobial effect of chitosan. Amount of chitosan addition was in inverse proportion to growth of *Leuconostoc* spp. and concomitant lactic acid production. At 4 °C fermentation, the population of CON was maximal (2.3 × 10^7 CFU/mL) in 14 d, whereas maximal populations of CS500 and CS1000 were 2.1 × 10^7 (in 21 d) and 1.1 × 10^7 CFU/mL (in 21 d), respectively. Subsequently, the population of *Leuconostoc* spp. decreased during the dongchimi fermentation. At 10 °C fermentation, the populations of CON (7.9 × 10^7 CFU/mL) and CS500 (1.0 × 10^7 CFU/mL) were maximal in 15 d whereas the maximal population of CS1000 (2.5 × 10^6 CFU/mL) appeared in 18 d. At 20 °C fermentation, maximal growth appeared in 4 d
and the populations of CON, CS500, and CS1000 were $7.5 \times 10^7$, $7.7 \times 10^7$, and $2.5 \times 10^7$ CFU/mL, respectively.

Addition of chitosan decreased considerably the growth of *Leuconostoc* spp. at the first viable cell count time (day 7 for 4 °C, day 3 for 10 °C, and day 1 for 20 °C) during the dongchimi fermentation. Addition of chitosan reduced initial population of *Leuconostoc* spp. due to its antimicrobial effect. Subsequently, the population of the chitosan-treated *Leuconostoc* spp. increased and finally their growth caught up with that in CON. After reaching the maximal growth, the populations were maintained and subsequently started decreasing. The acidity of the dongchimi increased due to the acid production by *Leuconostoc* spp. and concomitantly lowering pH provided an inhibitory effect, which decreased their population at final growth phase.

*Lactobacillus* spp. Figure 5(A), 5(B), and 5(C) show the growth profiles of *Lactobacillus* spp. at 4 °C for 49 d, at 10 °C for 21 d, and at 20 °C for 7 d, respectively, during dongchimi fermentation. Populations of *Lactobacillus* spp. in CON and AA300 increased exponentially over time and subsequently approached a plateau at approximately $10^7$ CFU/mL. On the contrary, populations of *Lactobacillus* spp. in CS500 and CS1000 decreased dramatically at the first viable cell count time and subsequently increased up to maximal growth. Thereafter, the populations were maintained or decreased at the late stage of the dongchimi fermentation. Chitosan treatment dramatically reduced initial population of *Lactobacillus* spp. due to its antimicrobial effect. The antibacterial capacity of chitosan on *Lactobacillus* spp. was more effective with increasing chitosan concentration.

![Graphs](image-url)
Control of dongchimi fermentation...

At 4 °C fermentation, the population of CON approached a maximal plateau ($6 \times 10^7$ CFU/mL) in 49 d. The populations of CS500 and CS1000 were maximal at $3.5 \times 10^7$ (in 35 d) and $2 \times 10^7$ CFU/mL (in 28 d), respectively. Subsequently the population of *Lactobacillus* spp. decreased up to approximately $10^6$ CFU/mL. At 10 °C fermentation, the populations of CON, CS500, and CS1000 were $6.5 \times 10^7$ CFU/mL, $2.7 \times 10^7$, and $1.8 \times 10^7$ CFU/mL, respectively, in 21 d. At 20 °C fermentation, a similar growth pattern was observed.

Excessive growth of *Lactobacillus* spp. affects negatively in the dongchimi fermentation. The growth period to reach the maximal population of *Lactobacillus* spp. is longer than that of *Leuconostoc* spp. since *Lactobacillus* spp. can grow and survive in the acidic environments rather than *Leuconostoc* spp. Finally, excessive growth of *Lactobacillus* spp. provides over-sourness of the dongchimi juice and lowers sensory quality such as taste and smell.

Addition of chitosan retarded excessive growth of *Lactobacillus* spp. in the dongchimi fermentation. The NH$_3^+$ groups on the chitosan molecules interact with the negative charges on the surface of the lactic acid bacteria including *Lactobacillus* spp. and *Leuconostoc* spp. (Liu and others 2004). The growth of the lactic acid bacteria is inhibited since the positively charged chitosan molecules cover the lactic acid bacteria, thereby interfering their proliferation (Rabea and others 2003; Gil and others 2004). The addition of chitosan has a potential to prolong the quality of the dongchimi during storage.

**Conclusions**

Chitosan can be used as a functional ingredient in a number of commercial food applications since the physicochemical properties of chitosans with certain degree of deacetylation and molecular weight can be tailored. In particular, the antibacterial activity of...
the chitosan is useful in the kimchi fermentation since the quality of kimchi can be prolonged by the simple addition of chitosan. Moreover, chitosan can be used for a variety of foods whose short shelf life or over-ripening is a problem.

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References


