Thesis for the Degree of Master of Science

Change of Pathogen Bacteria by Salting, Washing and Fermentation in *Kimchi*

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Pukyong National University

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김치제조시 염장,세척방법 및 발효에 따른 병원성 세균의 변화

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By

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요 약

김치는 전통적인 한국 발효 음식으로써 국제적 식품으로 발전하고 있지만 현대 사회에서 식품안전은 이미 불가피한 과제이가 되고있다. 김치는 살균과정이 없는 제품으로서 염장, 세척방법, 발효과정 시 유해미생물을 조절할 수 밖에 없다. 따라서, 이런 김치제조공정 중 제조조건에 따라 유해미생물에 미치는 영향을 조사하기위하여 4절배추를 대상으로 실험하였으며. 생 배추와 절인 배추에 각각 식중독 미생물인 *Escherichia coli* KCTC 1682, *Staphylococcus aureus* KCTC 1927 그리고 *Salmonella typhimurium* KCTC 1925을 인위적으로 접종하여 절임, 세척, 발효과정 중 유해미생물에 미치는 영향을 조사하였다.

4가지농도의 소금물(8%, 10%, 12%, 15%)로 절임실험을

진행한 결과, *Salmonella typhimurium* KCTC 1925는 소금물에 매우 민감하기 때문에 5시간만의 절임 후 4가지 소금물농도 모두에서 *Salmonella typhimurium*을 검측해낼 수 없었다. 이와 반대로, *Escherichia coli*와 *Staphylococcus aureus*는 NaCl에 아주 강한 저항성을 가지고 있어 15시간의 절임시간 후에도 선명하게 감소함을 관찰할 수 없었으며 8%, 10%, 12%, 15%의 소금물은 각각 *Escherichia coli*의 량을 4.40×10^{6} cfu/g으로부터 5.20×10^{5},1.64×10^{5} , 2.92×10^{5} , 1.98×10^{5} cfu/g로 감소되게 하였다. 또한, 8%,10%,12%,15% 소금물은 *Staphylococcus aureus*의 량을 2.02×10^{6} cfu/g으로부터 각각 1.9×10^{4} cfu/g , 4.1×10^{3} , 1.48×10^{3} , 6.65×10^{4}cfu/g로 감소하게 하였다.

10% 소금물에 15시간 절인 후 네 가지 세척방법 (2회 물세척, 3회 물세척, sodium dichlroisocyanurate (NaDCC)로 3회 세척, 3%초산으로 3회 세척)으로 4절배추를 세척하였다. *Staphylococcus aureus*는 4 가지 세척방법으로 완전히 제거할 수 있으나 NaDCC와 초산으로의 3회 세척하여 더 좋은 제거 효과를 얻었으며, *Escherichia coli*는 절임배추에서 발견되지 않음을 확인하였다. 발효실험에서 *Escherichia coli*는 pH값이 3.63이고 산도가

1.16%에 도달했을 때 김치에서 발견되지 않았으며 이와 다르게 *Staphylococcus aureus*는 갓 숙성되기 시작하였을 때 완전히 억제되었으며 pH값이 4.14보다 작고 산도가 0.73%에 도달하였을 때 김치에서 검출해내지 못하였다. *Staphylococcus aureus*와 비슷하게 *Salmonella typhimurium*도 pH값과 산도가 각각 4.09와 0.77%에 도달하였을 때 김치에서 발견할 수가 없었다. 김치 숙성과정에서 *Escherichia coli*가 제일 높은 산 저항능력이 있음을 확인하였고, 유해 미생물의 감소와 함께 유산균은 증가함을 확인 할 수 있었다.

Introduction

Kimchi is the general term given to a group of fermented vegetable food in Korea. Records indicate that salted vegetables as a type of macerated vegetable were consumed in Korea as early as the 3rd or 4th century A.D.

Most vegetables cultivated in Korea such as Chinese cabbage, radish, ponytail radish, young oriental radish, cucumber, green onion, lettuce, western cabbage, leek, green pepper, etc. are used as sources for making *kimchi*. Although 161 or 187 kinds of *kimchi* are currently reported, depending on the varieties and preparation methods of those vegetables, baechu *kimchi* and kaktugi are the most favored in Korea at present. The basic steps of preparing *kimchi* include salting vegetables, washing the salted vegetables with fresh water, adding spices and seasonings such as red pepper and garlic, and finally keeping the seasoned vegetables in cool place for a few days. *kimchi* has its best flavor, taste, and texture when optimally fermented at about pH 4.2; after optimum fermentation, *kimchi* quality deteriorates rapidly due to formation of excessive organic acids and texture softening (Park and Cheigh, 2004; Park and Cheigh, 1994; Kim, 2003).

Kimchi has been scientifically proved to be highly nutritious and recommended as a future food by many nutritionists at home and abroad. So the export of *kimchi* to foreign countries is rapidly increasing. Korean immigrants to China, Russia, Hawaii and Japan first introduced *kimchi* abroad, and have continued to eat *kimchi* as a side dish(Korea tourism organization).Gradually, besides Korea immigrants, *kimchi* were consumed by foreigners generally. Accordingly, *kimchi* may be found wherever Koreans live. Especially in America and Japan where relatively many Koreans live, packed *kimchi* is easily available. In the past, the production and consumption of *kimchi* was confined to Korean societies, but nowadays it has become a global food. And a lot of *kimchi* process factories were founded in abroad, specially in China.

Soil and water are always considered as the main contamination sources of pathogen microorganisms contained by fresh vegetables. Soil and water can be carriers of many microorganisms including pathogenic strains of *Escherichia coli*, *Salmonella* spp., *Vibrio cholera*e, *Shigella* spp.,*Cryptosporidium parvum*, *Giardia lamblia*, *Cyclospora cayetanensis*, *Toxiplasma gondii*, and the Norwalk and hepatitis A viruses. Even small amounts of contamination with some of these organisms can result in food-borne illness. Research has shown that the use of contaminated irrigation water can increase the frequency of

pathogen isolation from harvested produce (Norman and Kabler, 1953; Dunlop and Wang, 1961; Jones, 1999;Gagliardi and Karns, 2002),for this reason, as the major material, cabbage is easy to be contaminated by pathogen microorganisms. In the meanwhile, it is well known that the nasopharynx and skin of man are the most common reservoirs of *Staphylococcus aureus*. for this reason, foods like *k*imchi that are hand processed are especially prone to contamination with *Staphylococcus aureus*(Simone *et al*., 1982).

Brining and rinsing as the most vital steps during *kimchi* process will deeply influence the taste, texture, fermentation and preservation of final product. Brining extract the water from raw materials by osmotic activity and suppresses the growth of some undesirable bacteria that could spoil the *kimchi* ingredients. At the same time, it makes conditions relatively favorable for lactic acid bacteria by increasing the salt in the cabbage. In general for baechu *kimchi*, brining is carried out over a wide time range from 1 to 15 hours, depending on the salt concentration from 8 to 18 percent and salting temperature from 8 to 25℃, and the salt concentration of final product is adjusted to 2.2 to 3.0 percent through rinsing. (Han and Noh, 1996; Park and Jang, 2000; Shim *et al.*, 2003; Kim, 1997). Meanwhile, in the processes of salting, the reduction of food-borne bacteria could be observed, which mainly due to the existing

of high concentration of NaCl. NaCl has been proved as an effective bactericide (Hajmeer *et al*., 2006; Hajmeer *et al*., 2004;Gibson and Roberts, 1986; Lee *et al*., 2003; Jay, 1996).

Rrinsing added some disinfections such as acetic acid and chlorine can eliminate most of food-borne pathogens effectively, organic acids such as acetic, lactic, and citric have been employed at concentrations between 2% and 7% with variable results.

Chlorine is the most widely used sanitizing agent for reducing pathogens on whole and fresh-cut vegetables. The recommended concentrations of Cl range from 50 to 200ppm with a contact time of 1□ 3min (Beuchat, 1998; Cherry,1999).The main chlorine sanitizer that has long been recognized and has a high disinfection efficacy in the food industry is sodium hypochlorite (NaOCl) (Dychdala, 2001; Takano and Yokoyama, 2001).

Nevertheless, in recent years products based on the solid organo-chlorine compound sodium dichloroisocyanurate (NaDCC) have become increasingly popular because of their many advantages over sodium hypochlorite (Costaes, 1996; Clasen and Edmondson, 2006). Like other forms of chlorine, NaDCC produces hypochlorous acid, a well-known oxidizing agent. While both NaOCl and NaDCC rely on HOCl as the active agent, there are important differences in the performance of the

two compounds. Unlike NaOCl which releases all of its chlorine as free available chlorine, NaDCC releases only approximately 50% of the chlorine as free available chlorine, the balance remaining as ‘‘reservoir chlorine’’ (bound) in the form of chlorinated isocyanurates (Bloomfield and Miles, 1979). When the free available chlorine is used up, the equilibrium is disturbed, immediately releasing further free available chlorine from the ‘‘reservoir’’ until the total available is used up. the stabilized chlorine in NaDCC acts as a reservoir of HOCl which is rapidly released when the free available chlorine is depleted (Kuechler, 1997, 1999). NaDCC may also offer other advantages in terms of stability, safety, up-front cost and convenience.Some investigations have indicated significantly higher bactericidal capacity for NaDCC compared with NaOCl and increased resistance to inactivation by organic material. Moreover, NaDCC showed significantly higher activity than NaOCl solution against all bacterial species, in particular *E.coli* (Bloomfield and Miles, 1979; Bloomfield, 1973; Clasen and Edmondson, 2006).

Some pathogens such as *Staphylococcus aureus*, *Salmonella Enteritidis* and *Escherichia coli* O157:H7 could survive during the initial fermentation, but as the fermentation proceed, gradually over time, the

viability of pathogens will lose. That is because the bacteriocin-producing microbes lagged in the growth at the initial fermentation stage and antibacterial activity of the bacteriocin may therefore be limited, however , as fermentation time goes on, bacteriocin will be accumulated, and then, the sterilization effect get stronger. On the other hand, some researches showed that *E. coli* O157:H7, *Staphylococcus aureus* and *Salmonella Enteritidis* remained throughout the whole fermentation period, which suggested that the contamination of *kimchi* with *E. coli* O157:H7, *Salmonella Enteritidis,* or *Staphylococcus aureus*, at any stage of production or marketing could pose a potential risk (Caplice and Fitzgerald, 1999; Inatsu *et al*., 2004; Kim and Yoon, 2005; Kim *et al*., 2004). And despite the efforts of many agencies, industries, and people, outbreaks continue to occur, food safety has become a very serious problem.

On the basis of the materials and process method, *kimchi* was predestined to be contaminated by pathogen bacteria easily, microbiological quality control was vital and essential.

Materials and Methods

1. Materials

To prepare the *Kimchi*, Chinese cabbage, minced ginger, minced garlic, green onions, anchovy sauce, and red pepper were purchased at a local market in Busan, Korea, and then stored in a refrigerator at 4℃. M-ineral solar salt was also purchased at the local market, but stored in a room temperature.

2. Methods

2.1. Bacterial cultures and inoculum preparation

Escherichia.coli KCTC 1682, *Staphylococcus aureus* KCTC 1927, and *Salmonella typhimurium* KCTC 1925 were maintained in nutrient agar (Acumedia , Neogen, USA) slants at 4℃ until use. Before experiment, this three strains were inoculated into 100ml lactose broth (Acumedia , Neogen, USA), respectively, and then incubated at 37℃ with continuous shaking for 24 h at 100 rpm to obtain cells in early

stationary growth phase.

Culture inoculations were finished by different methods. For the salting and washing trials, 10ml of cultures above were inoculated into 1.5 L of brine before 600g cabbages were submerged into the brine, respectively. For *kimchi* ripening trials, 10ml of cultures above were inoculate into 128g seasonings via mixing, and then spread the seasonings inoculated previously on the salted cabbages surfaces.

2.2. Cabbage treatment

For salting trials, Chinese cabbages were trimmed, washed, and then cut lengthwise into four parts with a knife inserted through the bottom of the cabbage head. One part of cabbage (600g) was soaked in 1.5L brine inoculated by food-borne bacteria. Four kinds of brine with different salt concentrations (8%,10%,12%,and 15%) were used to salt cabbage The salinity and account of food-borne bacteria were determined every five hours.

For washing trials, Chinese cabbages were trimmed, washed, and then cut lengthwise into four parts with a knife inserted through the bottom of the cabbage head. One part of cabbage(600g) was soaked in 1.5L of 10% brine inoculated with food-borne cultures for 15h at 20℃,

and then washed in a plastic cask containing 5L various solutions—tap water, 3% acetic acid, and 100ppm sodium dichloroisocyanurate solution(NaDCC). After washing, the salted cabbage was drained for 2h by gravity.

For ripening trials, the same salting method was used except inoculation with food-borne cultures, and then wash the salted cabbage with 5L tap water for three times in a plastic cask, followed by gravity draining to adjust the salt concentration to 2.5%. *Kimchi* was prepared on the basis of the following compositions: Chinese cabbage 100g, red pepper 4g, liquefied anchovy sauce 8.5g, chopped green onion 4g, minced garlic 4g, and minced ginger 1g.

2.3. Measurement of salinity of cabbage

Salted cabbage was washed in a plastic cask containing 5L tap water by 3 strokes, and then drained for 2 hours by gravity. Immediately after, the drained salted cabbage was homogenized using a Polytron homogenizer (Ultra-Turrax® T25 Basic, Germany), and filtered with gauze. The salinity of filtrate was determined by salt meter(Lutron YK-31SA, Taiwan)

2.4. Measurement of pH and titratable acidity

The cabbage tissue and *Kimchi* juice were homogenized together using a Polytron homogenizer (Ultr-Turrax® T25 Basic, Germany), and filtered with a filter paper (Quantitative ashless No 5A, Advantec). The pH and titratable acidity of the filtrate were determined using a pH meter and via the pH-metric method, respectively (Kim *et al*., 2000).

Total acidity was expressed as a content of lactic acid (weight %) by measuring the titration volume of 0.1N-NaOH (f=1.003) to adjust pH at 8.2.

$$\text{Total acidity (\%)} = \frac{ml \quad of \quad 0.1N - NaOH \times f \times 0.009}{\text{sample weight}} \times 100$$

2.5. Microbiological analysis

Before analysis, 10g of salted cabbage or *kimchi* were homogenized for 30s with 90ml of 0.85% sterile saline, using a stomacher (Easy mix, AES Laboratoire, France). Serial dilutions were performed as required.

For detection of *E.coli*, 1ml of serial dilution was cultured on Petrifilm™ E. coli and Coliform count plate (EC)(3M microbiology, USA) and incubated for 24h at 37℃. For detection of *Staphylococcus aureus*, 1ml of serial dilution was cultured on Petrifilm™ Staph Express Count Plate (STX) (3M microbiology, USA) and incubated for 24h at

37℃. For detection of *Salmonella typhimurium*, 0.1ml of serial dilution was spread on Bismuth Sulfite Agar (Bacto, Difco, USA) and incubated for 48h at 37℃.

For enumeration of lactic acid bacteria, 0.1ml of serial dilution was spread on BCP plate count agar (Eiken Chemical Co., Ltd.,) and cultured for 48h at 37℃.

Results and discussion

1. The change of *Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium* during salting

Escherichia coli, *Staphylococcus aureus* and *Salmonella typhimurium* were inoculated into cabbages with the concentrations of 4.40×10^6 cfu/g, 2.02×10^6 cfu/g, and 6.19×10^6 cfu/g, respectively.

Four kinds of brine (8%, 10%, 12%, and 15%) were used to salt cabbages, when cabbage was salted in 8% brine, after salting for 15 hours, the salinity of cabbage reached to 2.22%, and 0.92 log reduction in *Escherichia coli* numbers and 2.03 log reduction in *Staphylococcus aureus* numbers were observed, respectively (Fig.1).when cabbage was salted in 10% brine, after salting for 15 hours, the salinity of cabbage reached to 3.04%, and 1.43 log reduction in Escherichia coli numbers and 2.70 log reduction in *Staphylococcus aureusnumbers* were observed, respectively (Fig.2). when cabbage was salted in 12% brine, after salting

for 15 hours, the salinity of cabbage reached to 3.45%, and 1.17 log reduction in Escherichia coli numbers and 3.14 log reduction in *Staphylococcus aureus* numbers were observed, respectively (Fig.3).

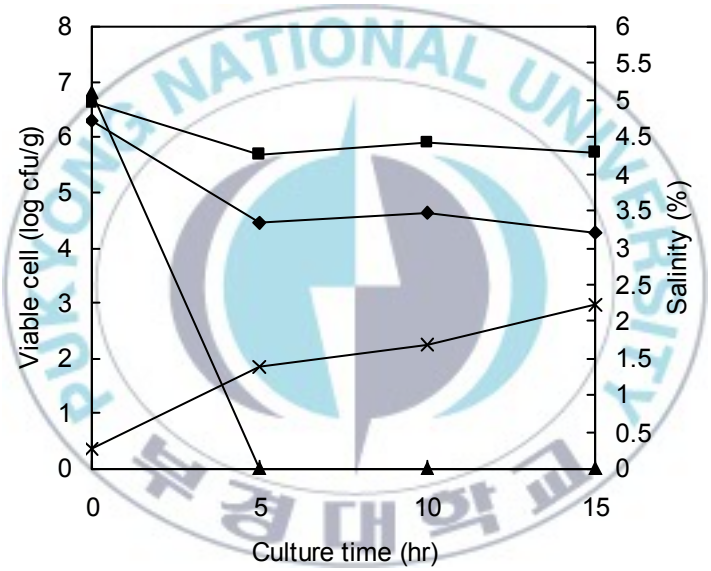

Fig. 1. Changes of pathogen bacteria and salinity during salting in Chinese cabbage with 8% brine

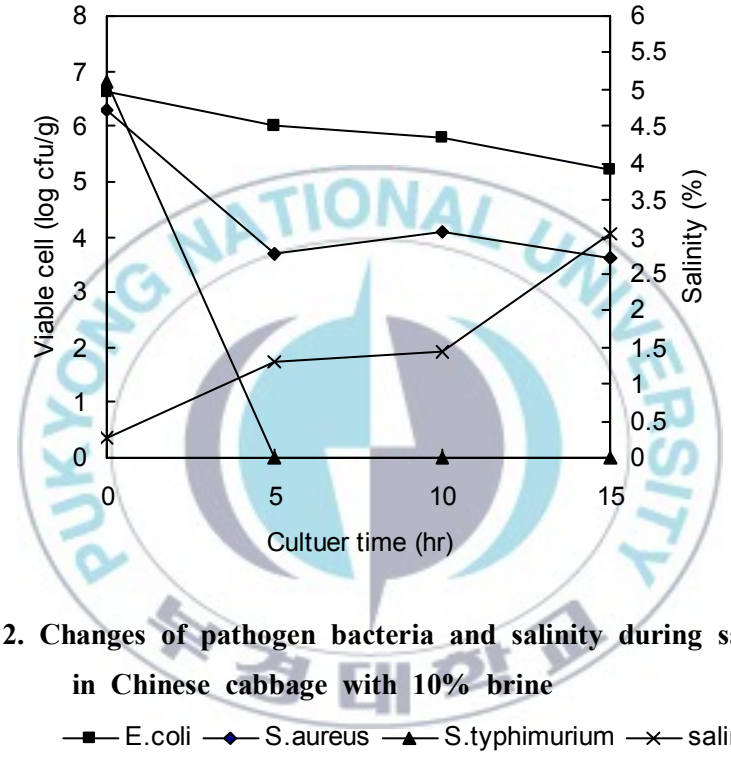

Fig. 2. Changes of pathogen bacteria and salinity during salting in Chinese cabbage with 10% brine

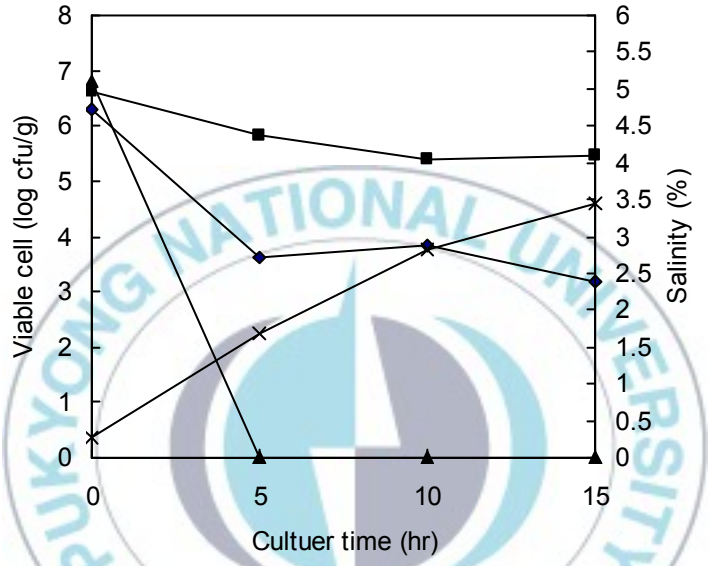

Fig. 3. Changes of pathogen bacteria and salinity during salting in Chinese cabbage with 12% brine

when cabbage was salted in 15% brine, after salting for 15 hours, the salinity of cabbage reached to 3.89%, and 1.34 log reduction in *Escherichia coli* numbers and 1.49 log reduction in *Staphylococcus aureus* numbers were observed, respectively (Fig.4). Though the amount of *Escherichia coli* and *Staphylococcus aureus* were not decreased obviously after 15h salting in all the four kinds of brine, by contrast, *Salmonella typhimurium*, which was very sensitive to brine, could not be detectable only after 5h salting in all the four kinds of brine (Fig.1,2,3,4).

In the processes of salting, the reduction of food-borne bacteria was mainly due to the existing of high concentration of NaCl. Because the amounts of NaCl and water are equal on both sides of the cell membrane, water moves across the cell membranes equally in both directions. When microbial cells are suspended in, asy, a 5% saline solution, the concentration of water is greater inside the cells than outside (concentration of H_2O is highest where solute concentration is lowest). The result to this case, water passes out of the cells at a greater rate than it enters. The result to the cell is plasmolysis, which results in growth inhibition and possibly death (Jay, 1996). *Staphylococcus aureus* was not eliminated entirely, which maybe mainly because of the high salt tolerance of *Staphylococcus aureus*. Some researches showed

Staphylococcus aureus can grow well in liquid medium with 11% NaCl

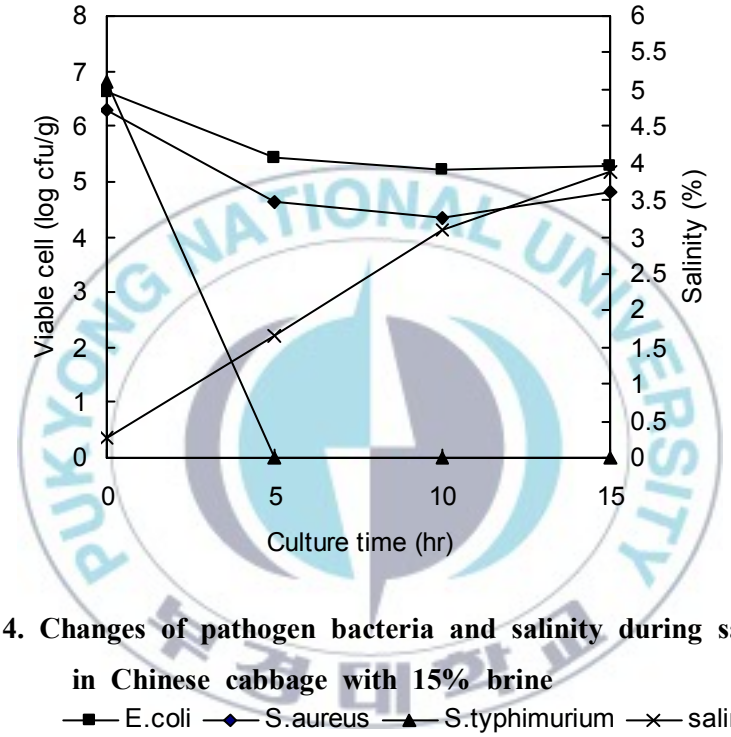

Fig. 4. Changes of pathogen bacteria and salinity during salting in Chinese cabbage with 15% brine

concentration, even in some literatures, where salt tolerance level of up to 20% for some strains of *Staphylococcus aureus* were reported (Jay, 1996; Lee et al., 2003; Shin et al., 1998).

The osmotolerance of *Staphylococcus aureus* to NaCl is portrayed in its ability to maintain its outer membrane structural integrity. *Staphylococcus aureus* can cope with osmotic environments by accumulating osmoprotectants such as proline and glycinebetaine in the cells under osmotic stress (Neidhardt *et al*., 1990)

Unlike the *Staphylococcus aureus*, the *Salmonella typhimurium* is unable to tolerate high salt concentration, brine above 9% is reported to be bactericidal (Jay, 1996).The growth of *salmonella* is generally inhibited in the presence of 3-4% NaCl. NaCl can bind free water necessary for *Salmonella* growth, which leads to decrease in water activity. The water activity for *Salmonella* growth is more restricted when compared to other bacteria and molds that exhibit minimum water activity values of 0.75 and 0.85, respectively.(Doyle, 1989).So *Salmonella typhimurium* could not be detected only after 5 hours salting.

The influence of NaCl on the growth of enteropathogenic *E. coli* has been studied in many researches (Hajmeer *et al.*, 2006; Hajmeer *et al.*, 2004;Gibson and Roberts, 1986; Lee *et al*., 2003), NaCl can inhibit

the growth of *Escherichia coli*, but on the other hand, the certain NaCl resistance of enteropathogenic *E. coli* have been determined.

E. coli O157:H7 was less tolerant to NaCl compared to *S. aureus* at both 5% and 10% NaCl concentrations. As NaCl concentration was increased from 5% to 10% for both salt grades/types the extent of cellular damage was bolstered (Hajmeer *et al.*, 2006). *E. coli* 0157:H7 is inhibited by NaCl in TSB at concentrations of ≥8.5% (Glass *et al.*, 1992).

Studies I have done revealed that *Escherichia coli* was inactivated gradual death was observed in 8, 10, 12, and 15% brine, but the reduction of *Escherichia coli* was relatively slow. Similar results were obtained by other researchers, when *Escherichia coli* was inoculated into trypticase soy broth that was adjusted to different NaCl concentrations, 2-log10-cfu /ml reduction within 96 h was observed in tryticase soy broth with 8.5% and 10.5% NaCl (Glass *et al.*, 1992). for enteropathogenic E. coli, which failed to grow at NaCl concentrations of >8% regardless of incubation temperature or pH (Gibson and Roberts, 1986).

In addition, the quality of brine will be changed during salting. The salinity of brine was lowered about 0.4-0.9% point after one time brine salting, and the pH decreased from 8.0 to 6.3 (Han *et al.*, 1998),

moreover soluble solid content and chemical oxygen demand of brine will be changed during salting (Yoon *et al*., 1999) ,which maybe also influence the ability of brine to kill the *Escherichia coli*.

According to the results obtained in this research, 15% brine isn't as effective as 12% brine did on inhibition bacteria, which is possible. The inhibition effect of sodium chloride on the growth o f bacteria is very complex, Chloride salts may have at least six de tr-imental effects on microorganisms: (1) increasing the osmotic pre s-sure which brings about plasmolysis; (2) decreasing the water act i-vity; (3) yielding the harmful chloride ion (4) reducing the solubi l-ity of oxygen; (5) sensitizing the cell to carbon dioxide; and (6) interferingwith the action of proteolyticenzymes (Frazier and West hoff, 1988). So higher concentration maybe not always more effecti -ve than the low concentration.

2. The change of *Escherichia coli, Staphylococcus aureus* and *Salmonella Typhimurium* during washing

After 15h of soaking in the 10% brine, four kinds of washing method were used to wash salted cabbages inoculated with food-borne bacteria before salting, the count of *E.coli* adhering to the salted

cabbages was reduced into1.20×10^5 cfu/g, 1.52×10^4 cfu/g, 4.55×10^5 cfu/g, and 0 cfu/g after water 2 strokes, water 3 strokes, NaDCC 3 strokes, and acetic acid 3 strokes, respectively. In the case of *Staphylococcus aureus*, residual counts were 3.05×10^5 cfu/g, 3.60×10^5 cfu/g, 5.65×10^3 cfu/g, and 7.70×10^3 cfu/g, respectively. Meanwhile, the same result that *Salmonella typhimurium* was undetectable was obtained after washed by any of the four kinds of washing methods (Table 1).

Though *Staphylococcus aureus* couldn't be eliminated entirely by any of the four kinds of washing methods, compared the four methods, NaDCC and acetic acid 3 strokes were more effectively to eliminate *Staphylococcus aureus*. In addition, The results in other study indicate that commercial rice vinegar (5.0% acetic acid) could reduce 3 log population of *E. coli* O157:H7 in iceberg lettuce at 25 ℃, while low concentration of vinegar (0.5% acetic acid and 0.05% acetic acid) was not found to exhibit any antimicrobial effect(Chang and Fang, 2007).

The early study suggested that using 5.0% (v/v) acetic acid for 5 min at 21 ℃ can reduce the population of *Shigella sonnei* on parsley more than 6 logCFUg-1 (Wu *et al*., 2000).

The previous study, parsley treated with vinegar containing ≥2.6% acetic acid noticeably discolored and had a strong vinegar odor(Wu *et al*.,

Table 1. Changes of pathogen bacteria according to different washing methods

Washing method	Population of food-borne bacteria		
	Escherichia coli (cfu/g)	*Staphylococcus aureus* (cfu/g)	*Salmonella typhimurium* (cfu/g)
Control[a)]	7.62×10^{6}	7.60×10^{6}	1.67×10^{6}
Water 2 strokes[b)]	1.20×10^{5}	3.05×10^{5}	0
Water 3 strokes	5.05×10^{4}	3.60×10^{5}	0
NaDCC[c)] **3 strokes**	2.54×10^{4}	5.65×10^{3}	0
Acetic-acid 3 strokes	2.33×10^{4}	7.70×10^{3}	0

a) Control means inoculation concentrate

b) One stroke means that spread the Chinese cabbages by means of inserting the fingers into the cabbages, and submerge cabbages into the water, then move from water surface to bottom, and followed by moving from plastic cask's left side to right side, and finally take the cabbages out of water.

c) NaDCC means sodium dichloroisocyanurate

2000). Moreover , the addition of rice vinegar containing 5% acetic acid may give lettuce an unacceptable sour flavor; however, washing with tap water will improve the unacceptable flavor(Chang and Fang, 2007).

Various theories have been put forth to explain the germicidal effects of chlorine. These include oxidizing the germ cells, altering cell permeability, altering cell protoplasm, inhibiting enzyme activity, and damaging the cell DNA and RNA. Chlorine appears to react strongly with lipids in the cell membrane, and membranes having high lipid concentrations appear to be more susceptible to destruction. For this reason, viruses, cysts, and ova are more resistant to disinfectants than are bacteria (Environmental Protection Agency, 1999).

Some researches found that at concentrations of 200 ppm, NaDCC yielded superior results compared to NaOCl and certain other agents used to sanitize fresh vegetables against aerobic mesophiles, molds and yeasts, total coliforms, *E. coli* and *Salmonella* sp. (Nascimento *et al*., 2003).

The effects of chlorine concentration on aerobic microorganisms and faecal coliforms present on leafy salad greens was studied, Populations of pathogens were markedly reduced with increased concentrations of chlorine to 50 ppm, but further increases in concentration to 200 ppm did not have a substantial additional effect

(Mazollier Jr, 1988).

3. The change of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella Typhimurium* during *kimchi* ripening

Before fermentation, *E.coli*, *S. aureus*, and *Salmonella* were inoculated into salted cabbages with the concentration of 7.90×10^6 cfu/g, 4.20×10^6 cfu/g, and 1.20×10^4 cfu/g, respectively. As ripening at 20 ℃ goes on, pH gradually lowed corresponding to titratable acidity increase. In the early stage of ripening, the account of *E.coli* increased gradually, but as the ripening proceeded, when pH lowered below 4.00 and acidity exceeded 1%, the account of *E.coli* decreased sharply, when pH 3.63 and acidity 1.16% have been reached, *E.coli* was undetectable in *kimchi* (Fig .5). Differently, from just beginning of ripening, the growth of *S. aureus* was inhibited, when pH lowed to 4.14 and acidity increased to 0.73%, the growth of *S. aureus* was inhibited entirely, no *S. aureus* could be detected in *kimchi* (Fig .6), and the similar result was observed in other study(Park, *et al.*, 2000). As same as to *S. aureus*, *Salmonella typhimurium* couldn't be detected as pH and acidity reached 4.09 and

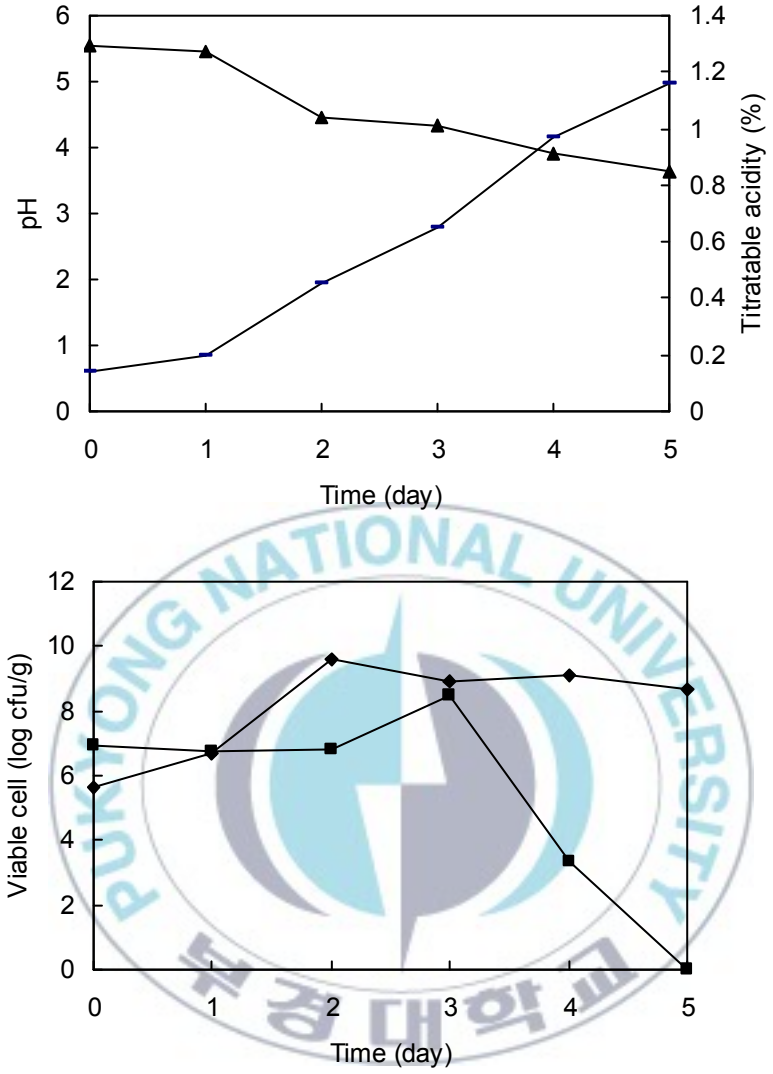

Fig. 5. Changes of pH, titratable acidity, lactic acid bacteria and *Escherichia coli* in *kimchi* during fermentation at 20℃

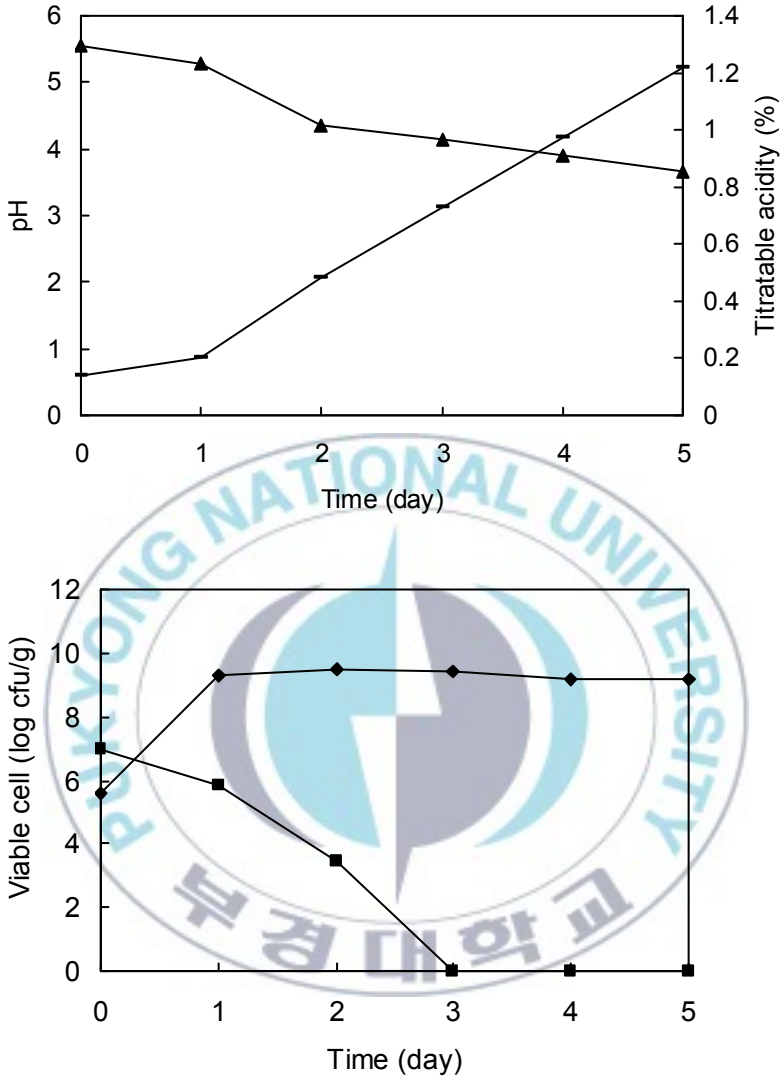

Fig. 6. Changes of pH, titratable acidity, lactic acid bacteria and *Staphylococcus aureus* in *kimchi* during fermentation at 20℃

0.77%, respectively (Fig .7). In previous studies, they reported that the lowest growth pH of *E.coli*, *S. aureus*, and *Salmonella typhimurium* were 4.0~4.5, 4.2~4.5, and 4.1~4.5, respectively (Jay, 1996; Kang, 2002; Bergdoll, 1989; Chung and Goepfert, 1970; Frazier and Weathoff, 1988; Buchanan and Klawitter, 1992).

In addition, propionic acid produced by lactic acid bacteria during fermentation and NaCl obtained during cabbage salting can inhibit the growth of *Salmonella typhimurium*, and the previous study showed that the inhibitory activity of propionic acid and NaCl was greatly enhanced when propionic acid was combined with NaCl (Hinton ,1999).

The parameters of pH, aw, nutrient content, salinity,and temperature are all interrelated for salmonella, as they are for most other bacteria, the minimum growth pH of bacteria also depend on the acid used to lower the pH (Jay, 1996). Food-borne bacteria such *E.coli* can survive in the neighborhood of pH 4.2, at which *kimchi* has the best taste, so contamination protection of food-borne bacteria have to be considered.

Accompany with decrease of food-borne bacteria, the account of lactic acid bacteria increased ceaselessly. The fermentation is mainly initiated by lactic acid bacteria such as *L. mesenteroides* under normal anaerobic circumstances, where lactic acid, acetic acid, carbon dioxide, and ethanol are produced as major end products. *L.mesenteroideds*

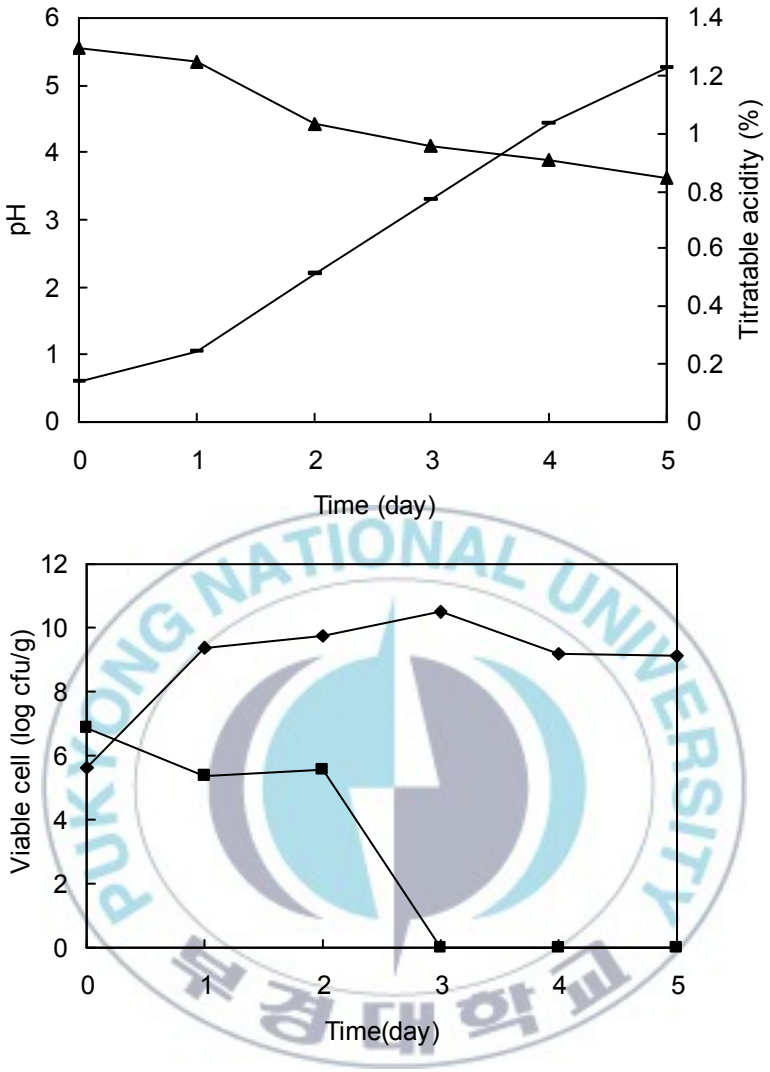

Fig. 7. Changes of pH, titratable acidity, lactic acid bacteria and *Salmonella typhimurium* in *kimchi* during fermentation at 20℃

is the predominant lactic acid bacteria in the early fermentation stages (Kim and Chun, 1996). As the pH drops to 4.6-4.9 with organic acid accumulation, *L.mesenteroides* is relatively inhibited, but the fermentation continues with other lactic acid bacteria such as *Streptococcus faecalis*, *Lactobacillus brevis*, *Pediococcus cerevisiae*, and *L.plantarum*. *L.plantarum* is present in the greatest numbers following the initial fermentation and gives the maximum acidity at the later stages. Lactic acid bacteria isolated from *kimchi* have antimicrobial activity against *E.coli*, *S. aureus*, *Salmonella typhimurium* and other microorganisms. Major lactic acid bacteria responsible for this activity are *Pediococcus cerevisiare*, *Leuconostoc* spp., and *L.plantarum*, and the properties of the inhibitory substances from these bacteria have been determined. These bacteria could produce adverse circumstances for the growth of other microorganisms (Park and Cheigh, 1994; Park *et al*., 1-983; Park and Song, 1991; Song and Park, 1992). Lactic acid content increases, but other nonvolatile organic acids contents are high at the beginning and decrease during fermentation. Acetic acid is the main volatile organic acid produced during *kimchi* fermentation. Heterofermentative LAB, such as *Leuconostoc mesenteroides* and *lactobacillus brevis*, could produce acetic acid (Park and Cheigh, 2004).

Escherichia coli has emerged extremely acid resistance during

kimchi fermentation, and the acid resistance also studied by other researchers. *E. coli* can use NaCl to counteract acidification of its cytoplasm by organic acids, and in addition, that combinations of antimicrobial agents cannot always be relied upon to achieve additive antimicrobial effects.The degree of protection observed in TSB-NaCl was dependent on the level of NaCl added; 0.5% NaCl led to a significant increase in survival and maximum protection was seen with the addition of 2% NaCl (Casey and Condon, 2002).

The widespread addition of NaCl to food also creates a need to elucidate the responses of potential pathogens to elevated NaCl concentrations. Responses to NaCl are also of considerable medical import because infecting organisms can be exposed to NaCl concentrations of at least 150 mM at various locations in the body (Rowbury *et a*l., 1996).

NaCl reduces the inhibitory effect of lactic acid on E. coliO157:H45 by raising its cytoplasmic pH. This protective effect is also seen with other strains of E. coli and with other organic acids (Casey and Condon, 2002). It is also plausible that the decreased aw due to the addition of NaCl contributed to the improved survival of the *E. coli* cells in acidified media (Meury, 1988).

When pH lowered below 4.32 and acidity exceeded 0.9%, the

amount of *E.coli* decreased sharply (Fig .5). the similar results were observed in other study, *E. coli* cells grown in TSB and challenged with organic acids (e.g. lactic, acetic and formic) at pH 4.2 died at a rapid rate (less than 0.01% survival after 2.25 h) (Clavero, and Beuchat, 1996).

Summary

Kimchi is a traditional, fermented Korean food, and gradually becomes a cosmopolitan food. In modern society, food safety has become an unevadable issue. In this paper, in order to determine the changes of foodborne bacteria by salting, washing and fermentation in kimchi, three kinds of food-borne bacteria including *Escherichia coli* KCTC 1682, *Staphylococcus aureus* KCTC 1927, and *Salmonella typhimurium* KCTC 1925 were inoculated into raw cabbages and salted cabbages, respectively.

For salting trials, four kinds of brine (8%, 10%,12%,and 15%) were used to salt cabbages, the amount of *Escherichia coli* and *Staphylococcus aureus* weren't decreased obviously after 15h salting in all the four kinds of brine, by contrast, *Salmonella typhimurium*, which was very sensitive to brine, couldn't be detectable only after 5h salting in all the four kinds of brine.

For washing trials, After 15h of soaking in 10% brine, four kinds of washing method(ie. water 2 strokes, water 3 strokes, sodium dichlroisocyanurate (NaDCC) 3 stokes, and acetic acid 3 strokes) were used to wash salted cabbages. Though *Staphylococcus aureus* and

Escherichia coli couldn't be eliminated entirely by any of the four kinds of washing methods, compared the four methods, NaDCC and acetic acid 3 strokes were more effectively to eliminate *Staphylococcus aureus* and *Escherichia coli.*

For fermentation trials, In the process of fermentation, when pH 3.63 and acidity 1.16% have been reached, *Escherichia coli* was undetectable in *kimchi*. Differently, from just beginning of ripening, the growth of *Staphylococcus aureus* was inhibited, when pH lowed to 4.14 and acidity increased to 0.73%, the growth of *Staphylococcus aureus* was inhibited entirely, no *Staphylococcus aureus* could be detected in *kimchi,* As same as to *Staphylococcus aureus*, *Salmonella typhimurium* couldn't be detected as pH and acidity reached 4.09 and 0.77%, respectively. *Escherichia coli* had the highest ability of acid resistance during *kimchi* ripening. Accompany with decrease of food-borne bacteria, Lactic acid bacteria performed an important role in eliminating pathogen bacteria. The amount of lactic acid bacteria reached to about 10^9cfu/g, and this was remained until the fifth day.

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