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Allergenicity Changes in Raw Shrimp (*Acetes japonicus*) and *Saeujeot* (Salted and Fermented Shrimp) in Cabbage *Kimchi* due to Fermentation Conditions

Jin-Gyu Park[2], Hiroki Saeki[1], Atsushi Nakamura[1], Koth-Bong-Woo-Ri Kim, Ju-Woon Lee[2], Myung-Woo Byun[2], Seong-Mi Kim[3], Sung-Mee Lim[4], and Dong-Hyun Ahn*

Faculty of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea
[1]Graduate School of Fisheries Science, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan
[2]Team for Radiation Food Science and Biotechnology, Advaned Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongup, Jeonbuk 580-185, Korea
[3]National Fisheries Products Quality Inspection Service, Busan 600-016, Korea
[4]Department of Food Science and Technology, Tongmyong University, Busan 608-737, Korea

Abstract *Saeujeot* (salted and fermented shrimp) and *kimchi* are traditional Korean fermented foods. Even though shrimp have often induced severe allergic reactions in sensitized individuals, few studies have investigated the allergenicity of shrimp. The aim of this study was to observe the changes of pH and allergenicity of raw shrimp (*Acetes japonicus*) and *saeujeot* in cabbage *kimchi* during fermentation using competitive indirect enzyme-linked immunosorbent assay (Ci-ELISA). Fermentation was carried out at different temperatures (25, 15, and 5°C). The pH of cabbage *kimchi* added with raw shrimp or *saeujeot* slowly decreased at lower temperature (5°C) at the end stage of the fermentation process. The binding ability of serum obtained from patients allergic to raw shrimp against shrimp tropomyosin and *saeujeot* in *kimchi* rapidly decreased during longer fermentation periods and higher temperature (25°C). In conclusion, the allergenicity of both raw shrimp and *saeujeot* in *kimchi* decreased during fermentation but the decrease in allergenicity of *saeujeot* was greater than observed for raw shrimp.

Key words: shrimp allergy, *saeujeot* (salted and fermented shrimp), *kimchi*, allergenicity

Introduction

Allergy reactions are immediate hypersensitivity reactions triggered by allergens that bind to IgE (1-3). The sequence of events in immediate hypersensitivity consists of three steps. The first step is the production of IgE and sensitization. When the allergen stimulates T and B cells, the B cells specific to the antigen are activated by T_H2 cells, and allergen-specific IgE is produced by the B cells. The allergen-specific IgE enters the bloodstream and binds to FcR receptors on tissue mast cells and basophils. These cells are then sensitized and poised to react to subsequent encounters with the allergen (4, 5). The second step of immediate hypersensitivity is the induction of mast cell degranulation. Mast cells are activated by the cross-linking of FcR receptors, which occurs via the binding of multivalent antigens to the attached IgE molecules. Activation of the mast cells results in the release of mediators, which include biogenic amines, lipid mediators, and cytokines (6). In the third step, these mediators cause an acceleration of capillary vessel permeability, smooth muscle contraction, and secretion acceleration (7). The most common allergic diseases are bronchial asthma, urticaria, allergic rhinitis, atopic dermatitis, allergic conjunctivitis, and allergic gastroenteritis. (8). Major allergens are mites, mold, pollen, antiseptics, cosmetics, drugs, and food. Food allergens are especially known to cause immediate hypersensitivity reactions (9). Among the commonly allergenic foods are eggs (10), soybean (11), milk (12), shrimp (13), mackerel (14), beef (15), etc. Food allergies occur in 4 to 6% of children, and in 1.5% of adults in Korea (16).

Kimchi is a common food produced by fermentation via lactic acid production at low temperatures. It is processed with a seasoning mixture consisting mainly of salt, red pepper powder, garlic, ginger, green onion, *saeujeot*, and radish (17, 18). *Saeujeot* is a fermented seafood product that is ripened primarily by proteolytic enzymes present in the shrimp. During proteolysis, flavor and aroma substances are formed and a characteristic consistency develops. The proteolytic products formed during fermentation are mainly composed of soluble nitrogenous compounds such as amino acids, peptides, nucleotides, and their decomposition products (19-21). Shrimp is one of the most allergenic foods known and has been shown to induce dermatitis, gastroenteritis, and bronchial asthma (22). The only major allergen identified in shrimp is the shrimp muscle protein tropomyosin of 36 kDa (23, 24). Previous research has investigated the changes in allergenicity of *saeujeot* at various salt concentrations and temperatures during fermentation, and reported that the binding ability between the allergen and a monoclonal antibody of tropomyosin decreased during the fermentation period (25). In this study, we investigated the allergenicity of serum obtained from patients allergic to shrimp against raw shrimp and *saeujeot* in *kimchi* using a competitive indirect enzyme-linked immunosorbent assay (Ci-ELISA).

*Corresponding author: Tel: +82-51-620-6429; Fax: +82-51-622-9248
E-mail: dhahn@pknu.ac.kr
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Materials and Methods

Shrimp and *saeujeot* The raw shrimp used in this study was *Acetes japonicus*. For preparing *saeujeot*, raw shrimp was combined with a 25%(w/w) salt concentration and fermented for 6 months at 20±2°C.

Standard antigen and antibody The standard antigen was shrimp tropomyosin obtained from the shrimp (*Fenneropenaeus chinensis*), which was supplied by the Radiation Food Science and Biotechnology Team at the Korea Atomic Energy Research Institute. The serum from patients allergic to shrimp was obtained from the medical department of Hokkaido University Hospital in Japan. The goat anti-human IgE/IgG conjugated peroxidase was purchased from Sigma Corporation (secondary IgE; Sigma Chemical Co., St Louis, MO, USA).

Experimental conditions of the Ci-ELISA The binding abilities of the protein solution and antibody were carried out according to the modified Ci-ELISA method of Lee *et al.* (26). Costar 96-well flat bottom plates (469957; Nunc, Kamstrupvej, Denmark) were coated with tropomyosin in 0.2 M biocarbonate coating buffer (pH 9.6) and incubated at 4°C, overnight. The wells were blocked for 2 hr with 0.01 M phosphate buffered saline (PBS, pH 7.3) containing 1% gelatin, and then 50 μL of dilute antigen and 50 μL of antibody were added to the wells, respectively. After the secondary antibody (goat anti-human IgE/IgG-peroxidase) was added, o-phenylenediamine (OPD, Sigma Chemical Co.) solution was used to measure the color for 30 min. After the reaction was stopped with 2 M H_2SO_4, the absorbance was measured at 490 nm using an ELISA reader (model 550; Bio-Rad, Hercules, CA, USA). After each reaction step was finished, the wells were washed 4 times using 0.01 M PBST (phosphate buffered saline containing 0.05%, v/v, Tween 20). All reactions were conducted at 37°C for 2 hr, with the exception of the coating.

Titration curve of Ci-ELISA A titration curve was made to define the optimum concentration combination of standard antigen (tropomyosin) and primary antibody (serum of shrimp-allergic patients) according to the modified method of Lee *et al.* (27). The standard antigen was diluted to the proper ratio with 0.2 M bicarbonate coating buffer (pH 9.6), loaded into the wells, and then coated overnight at 4°C in a refrigerator (SR-5844; Samsung, Youngin, Korea). The patient serum was then diluted in several ratios with 0.01 M PBS, and 100 μL were loaded into each well. All experiments were carried out by the Ci-ELISA course.

Standard curve of Ci-ELISA The wells were coated with tropomyosin diluted with 0.2 M biocarbonate coating buffer (pH 9.6). The antigen was diluted to concentrations ranging from 50 to 0.024 μg/mL with 0.01 M PBS (pH 7.3). Then, 50 μL of the dilute solution was added to each well. Fifty μL of the dilute concentration of antibody, as determined by the titration curve, was put into the wells. The courses and conditions for the experiments were the same as those of the Ci-ELISA. Each 50 μL of the antibody and 0.01 M PBS (pH 7.3) were added for 100% binding of the standard antigen and antibody. One-hundred μL of 0.01 M PBS (pH 7.3) was used as the blank.

Table 1. The formulation for *kimchi*

Ingredient	Ratio (%)
Salted Chinese cabbage	80.2
Radish	5.0
Powdered red pepper	3.5
Garlic	2.0
Ginger	0.5
Onion	1.0
Stone-leek	1.0
Leek (*Allium odorum*)	1.5
Saeujeot	2.0
Paste of glutinous rice	1.0
Sugar	0.3
Salt	2.0

Manufacture and fermentation of *kimchi* The mixing ratios of the *kimchi* formulation are shown in Table 1. Cabbage was washed and brined in an 8% salt solution for 18 hr, rinsed with fresh water, and drained for 4 hr. Spices were added at their proper ratios. In this study, *saeujeot* fermented in 25% salt solution for 6 months was added. Therefore, the amount of salt added to the *kimchi* was adjusted to correct for 25% salt concentration when adding the *saeujeot*. However, unlike *kimchi* added with *saeujeot*, a greater amount of salt was present in the *kimchi* with shrimp. *Kimchi* was fermented at 25, 15, and 5°C for 12 to 30 days, respectively.

Preparation of shrimp and *saeujeot* extracts Shrimp extracts were used to determine the changes of allergenicity in shrimp and *saeujeot* at different fermentation period. Two g of shrimp or *saeujeot* in *kimchi* were soaked in distilled water to remove foreign material such as the *kimchi* spices. Shrimp or *saeujeot* were then homogenized in 20 mL of 0.01 M PBS (pH 7.3). The homogenates were stirred overnight at 4°C and then centrifuged (9,000×g, 30 min, 4°C). The protein concentrations of the extracts were determined at a 1 mg/mL level using a BCA protein assay kit (BK43359; Pierce, Rockford, IL, USA). Also, the *kimchi* containing the added raw shrimp and *saeujeot* was used to evaluate the changes in allergenicity

pH measurement The pH values of *kimchi* juice with raw shrimp and *saeujeot* were measured using a pH meter (HM-30V; Toa, Tokyo, Japan).

SDS-PAGE Sodium dodecyl sulfate polyacryamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemmli (28) to determine the degree of tropomyosin decomposition during fermentation. Molecular weight (Mw) markers were purchased from BioLabs (P7702S;

New England BioLabs, Beverly, MA, USA). The Mw standards were insulin A and B chains (2.3 and 3.4 kDa, respectively), aprotinin (6.5 kDa), lysozyme (14 kDa), trypsin inhibitor (20 kDa), triosephosphate isomerase (26 kDa), lactate dehydrogenase (36 kDa), MBP_2 (42 kDa), glutamic dehydrogenase (55 kDa), serum albumin (66 kDa), phosphorylase b (97 kDa), β-galactosidase (116 kDa), MBP-β-galactosidase (158 kDa), and myosin (212 kDa). Shrimp or *saeujeot* taken from the *kimchi* were slightly rinsed to remove seasoning, and then homogenized in 0.01 M PBS (pH 7.3). Then, 40 mg/mL of the shrimp muscle protein was used as the SDS-PAGE sample. SDS-PAGE was performed with a 12% running gel and 4% stacking gel. Ten μL of sample solution was loaded into each well. To observe the separated band, the gel was stained with coomassie brilliant blue R250 (CBB), and destained with 5% methanol and 7% acetic acid solution. A scanner (Power Look III; Amersham Pharmacia Biotech Company, Piscataway, NJ, USA) was used to analyze the SDS-PAGE gel.

Statistical analysis All results were obtained from 3 independent experiments and averaged. Data were analyzed using the statistical analysis system software. Analyses of variance were performed by analysis of variance procedures. Significant differences ($p<0.05$) between means were determined Duncan's multiple range test.

Results and Discussion

Titration curve To trace the optimum dilution concentration combination of the standard antigen and first antibody (shrimp and serum of patients allergic to shrimp, respectively), the antigen was diluted to 10 μg/mL, and the antibody was diluted to various concentrations, and then a titration curve was constructed. At this stage, the antibody reacted very strongly with the dilute antigen at the concentration range from 0.063 to 0.016 μg/mL. Therefore, the optimal dilution concentration of the coating antigen was 10 μg/mL at 0.016 μg/mL of antibody (Fig. 1).

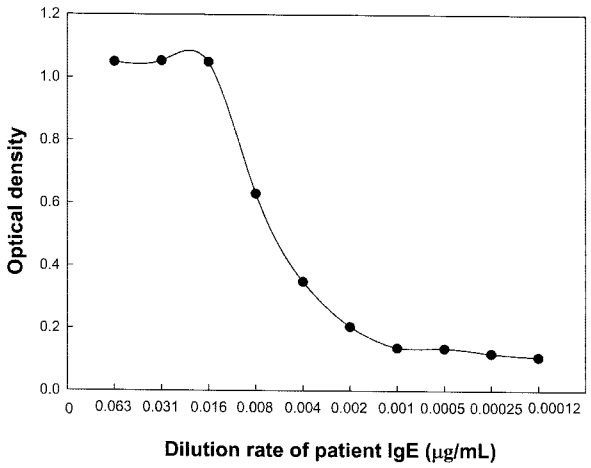

Fig. 1. Titration curve at different concentrations of serum from patients allergic to shrimp to tropomyosin using Ci-ELISA. Secondary IgE solution was diluted to 1:250 with PBS. Concentration of coated tropomyosin is 10 μg/mL.

Standard curve From the standard curve at 10 μg/mL of antigen and 0.016 μg/mL of serum from the shrimp-allergic patients, the concentration of tropomyosin reacted with the patient serum was calculated by the following:

$$x = e^{\left(\frac{0.502-y}{0.1516}\right)}$$

x=concentration of tropomyosin reacting with shrimp-allergic patients' serum
y=optical density (OD) value

The optimum concentration range of the reacting tropomyosin was 0.024 to 6.25 μg/mL (Fig. 2). The error range was $p \leq 1$.

The change in pH of *kimchi* with added shrimp and *saeujeot* The purpose of the *kimchi* fermentation process is to decompose and resynthesize its major components. In particular, carbohydrate is decomposed to organic acid,

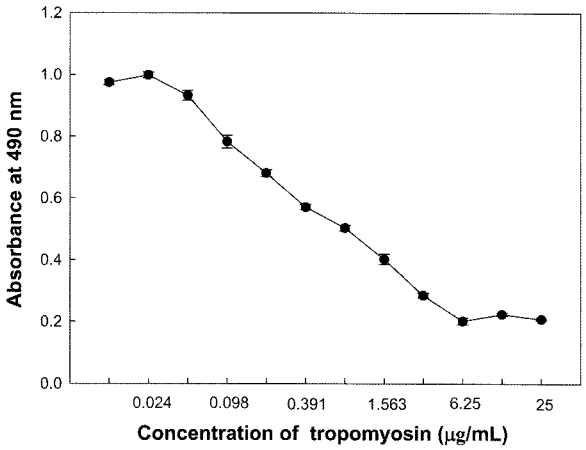

Fig. 2. Standard curve of serum from patients allergic to shrimp to tropomyosin by Ci-ELISA. Tropomyosin was serially double-diluted from 25 to 0.012 μg/mL. The range of optimal detection was from 0.024 to 6.25 μg/mL.

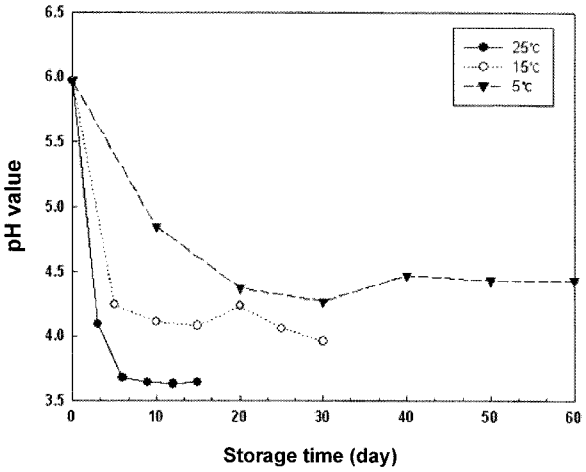

Fig. 3. pH changes in *kimchi* with raw shrimp at different fermentation period.

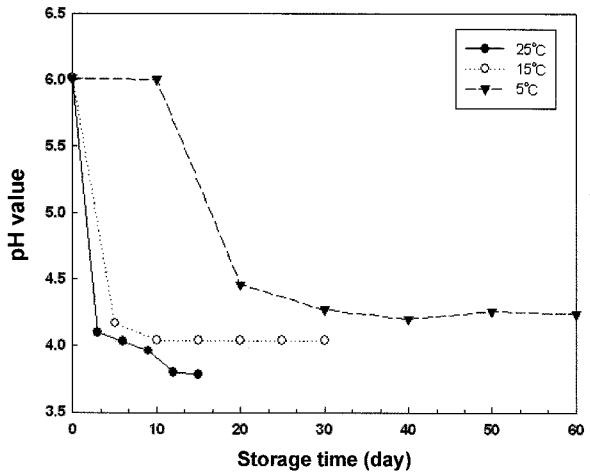

Fig. 4. pH changes in *kimchi* with *saeujeot* at different fermentation period.

giving *kimchi* a fresh sour taste. Thus, the pH and acidity of *kimchi* can be principal indicators of its quality (29). The pH of the cabbage *kimchi* added with shrimp slowly decreased during storage at low temperatures (Fig. 3). The change in pH of the *kimchi* with *saeujeot* during fermentation was similar to the results of the *kimchi* with added shrimp. However, its pH decreased rapidly during the early period of fermentation (Fig. 4). The optimum fermentation times of the *kimchi* with shrimp or *saeujeot* were similar, after approximately 3, 5, and 20 days at 25, 15, and 5°C, respectively. In general, the optimum fermentation pH for *kimchi* is in the range of 4.0-4.5 (30). In the present study, the optimal fermentation period of the *kimchi* with shrimp was about 3 days at 25°C, 5 days at 15°C and 20 days at 5°C, respectively. Lee *et al.* (31) reported that pH changes in *kimchi* during fermentation were correlated to its microorganism activity, taste, organic acid content, salt content, etc. Therefore, we assumed that the drop in pH was due to the production of lactic acid and acetic acid by lactic acid bacteria, and we also found that the pH decrease was faster at higher fermentation temperatures. Kang *et al.* (32) similarly reported that a slight change in pH during final period of fermentation was caused by the buffering action of free amino acids and inorganic ions in the *kimchi* juice.

Allergenicity change of shrimp in *kimchi* Shrimp and *saeujeot* are known to be allergy-causing foods. The major allergen of shrimp is its 36 kDa tropomyosin protein, which is stable to heat and enzyme action (33). After adding the shrimp to *kimchi,* we monitored the allergenicity change of the shrimp allergen at different *kimchi* fermentation conditions. According to Jin and Hong (34), the chloroplast protein decomposition of plants is caused by endopeptidase and aminopeptidase existing in the thylakoid membrane. Therefore, the proteolysis of cabbage might be caused by these two enzymes. After adding the shrimp to the *kimchi*, the degree of protein decomposition during fermentation was determined by SDS-PAGE. The tropomyosin band disappeared after 6 days fermentation at 25°C, 10 days at 15°C, and 30 days at 5°C. These results indicate that as

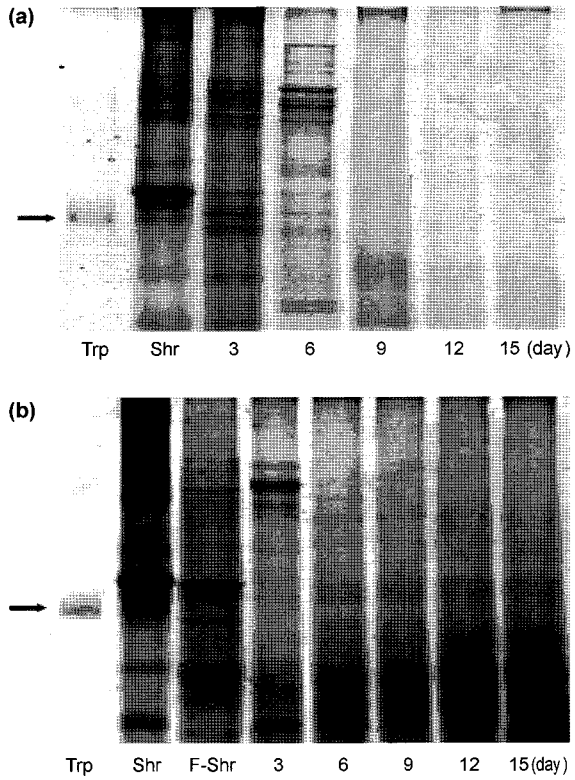

Fig. 5. Changes of tropomyosin in raw (a) and *saeujeot* (b) meat of *kimchi* at 25°C. Trp, tropomyosin; Shr, raw shrimp meat; F-Shr, *saeujeot* meat.

Table 2. Degradation time course of tropomyosin band in raw and salt-fermented shrimp meat of *kimchi* fermented at different temperatures

	Degradation time of tropomyosin band (day)		
	25°C	15°C	5°C
Raw shrimp	6	10	30
Salt-fermented shrimp	3	5	20

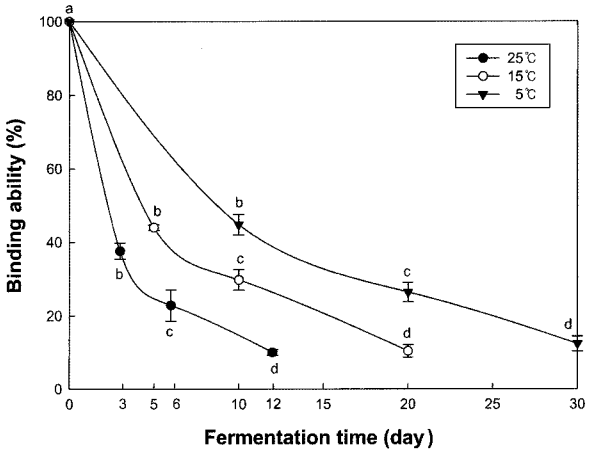

Fig. 6. Binding abilities of the allergen in raw shrimp meat of *kimchi* at different temperatures. The binding ability was measured by Ci-ELISA. Binding ability=Bt/Bo×100. Bt, binding ability of raw shrimp meat of *kimchi* to patients serum; Bo, binding ability of raw shrimp to patients serum.

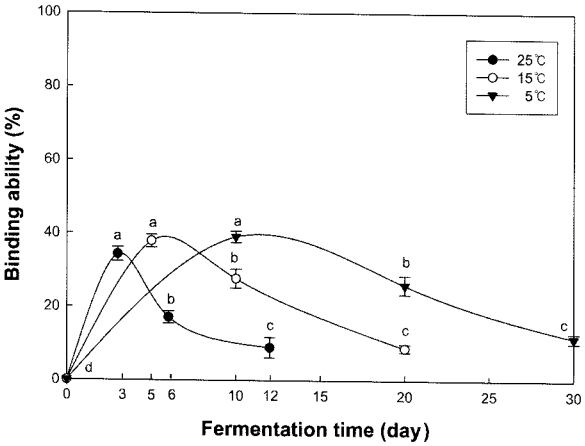

Fig. 7. Binding abilities of the allergen in juice of *kimchi* with raw shrimp at different temperatures. The binding ability was measured by Ci-ELISA. Binding ability=Bt/Bo×100. Bt, binding ability of *kimchi* juice to patients serum; Bo, binding ability of raw shrimp to patients serum.

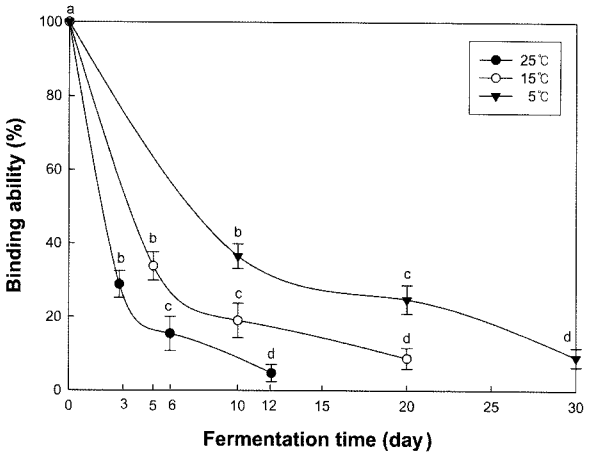

Fig. 8. Binding abilities of the allergen in the *saeujeot* meat of *kimchi* at different temperatures. The binding ability was measured by Ci-ELISA. Binding ability=Bt/Bo×100. Bt, binding ability of *saeujeot* meat of *kimchi* to patients serum; Bo, binding ability of raw shrimp to patients serum.

fermentation temperature increased, degradation speed also increased (Fig. 5, Table 2).

Also, as fermentation temperature increased, the binding activity between the shrimp allergen and the patient serum decreased (Fig. 6). Choi *et al.* (35) determined the degree of fermentation and quality changes of *kimchi* stored at 17 and 4°C, in which the *kimchi* stored at 17°C, total microorganisms and lactic acid bacteria increased, whereas the speed of reducing sugar accumulation and degree of acid production decreased. In addition, changes in sensory characteristics were rapid. These results were influenced by the effect of shrimp tropomyosin degradation via protein degradation (36). The binding ability of *kimchi* juice containing shrimp temporarily increased in the initial days of fermentation and then subsequently decreased (Fig. 7). Because salt-soluble tropomyosin was released from the shrimp muscle, binding ability increased during the early storage days and decomposed as passing fermentation time increased. In summary, when the pH reached an optimum level over a sufficient fermentation time (i.e., on 12 days at 25°C, 20 days at 15°C, and 30 days at 5°C), the shrimp tropomyosin was completely decomposed. Hence, allergy induction by *kimchi* juice and shrimp might no longer pose a problem even for allergic patients to shrimp.

Allergenicity change of *saeujeot* in *kimchi* There are three important enzymes that accelerate *saeujeot* fermentation: enzymes from shrimp viscera, shrimp meat (muscle), and microbial origins (37, 38). Microbes that exist in *saeujeot* include *Micrococcus* sp., *Brevibacterium* sp., *Sarcina* sp., *Leuconostoc* sp., *Bacillus* sp., *Pseudomonas* sp., *Flavobacterium* sp., and various yeasts which are involved in protein decomposition (39). The binding ability of the *saeujeot* allergen decreased with increasing fermentation time, and this decrease in binding ability was faster than that decrease in binding ability by raw shrimp added to *kimchi*. Using SDS-PAGE, the degree of protein degradation for the *saeujeot* from *kimchi* was determined by a tropomyosin band that disappeared after 3 days at

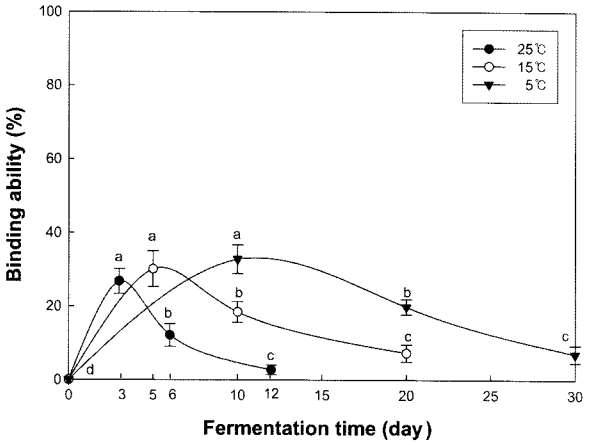

Fig. 9. Binding abilities of the allergen in juice of *kimchi* with *saeujeot* at different temperatures. The binding ability was measured by Ci-ELISA. Binding ability=Bt/Bo×100. Bt, binding ability of *kimchi* juice to patients serum; Bo, binding ability of raw shrimp to patients serum.

25°C, 5 days at 15°C, and 20 days at 5°C, respectively. As temperature increased, the degradation speed of tropomyosin also increased (Fig. 5, Table 2). The binding ability of the allergen in the *saeujeot* muscle from *kimchi* was well-maintained at low storage temperatures (Fig. 8). The change in allergenicity of the *kimchi* juice with *saeujeot* was similar to that of the *kimchi* with shrimp (Fig. 9). When the fermentation temperature was high, the binding ability was reduced quickly. Nam *et al.* (40) reported that protein degradation enzymes in *saeujeot*, a trypsin-like enzyme of the serine system (alkaline protease), showed the highest activity. Therefore, tropomyosin (the major allergen of shrimp) might be decomposed by an alkaline protease, and therefore the patient serum and tropomyosin almost didn't react. Similarly, Kim (41) and Bae *et al.* (42) reported that through the course of fermentation and enzyme hydrolysis, the major allergen of shrimp produced various low molecular nitrogen compounds, after which its

allergenicity became weaker. Barkholt *et al.* (43) reported that when peas were fermented with lactic acid bacteria at a moisture content of 70%, protein allergenicity decreased by 10%. Therefore, in *kimchi* with raw shrimp or *saeujeot*, allergenicity should decrease after 12 days of fermentation at 25°C, 20 days at 15°C, or 30 days at 5°C. During these periods, the allergic reaction typically induced by tropomyosin disappeared in the *saeujeot* and juice of *kimchi* because tropomyosin is completely decomposed at this optimum pH range (4.0-4.5). In the case of fermented shrimp, binding ability was reduced faster than in raw shrimp over the same period, because the *saeujeot* was fermented for 6 months and the tropomyosin was likely degraded by protease.

In conclusion, the binding ability of tropomyosin decreased faster in *kimchi* with *saeujeot* than *kimchi* with raw shrimp at higher fermentation temperature in the same period. But when considering stability and general food quality, *kimchi* made at low fermentation temperatures may prove to be a better food product. Tropomyosin in raw shrimp and *saeujeot* in *kimchi* was almost completely decomposed after reaching the optimum pH level (4.0-4.5) of *kimchi* so that the allergy induction potential from shrimp in *kimchi* is highly reduced.

Acknowledgements

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References

1. Kinet JP, Metzger H, Hakimi J, Kochan J. A cDNA presumptively coding for the alpha subunit of the receptor with high affinity for immunoglobulin E. Biochemitry 26: 4605-4610 (1987)
2. Siraganian RP, Hook WA, Levine BB. Specific *in vitro* histamine release from basophils by bivalent haprens: The evidence for activation by simple bridging of membrane bound antibody. Immunochemitry 12: 149-157 (1975)
3. Son DY. Research on the allergic potential of insecticidal cry1Ac proteins of genetically modified rice. Food Sci. Biotechnol. 15: 385-391 (2006)
4. Conard DH, Bazin H, Sehon AH, Foese A. Binding parameters of the interaction between rat IgE and rat mast cell receptors. J. Immunol. 114: 1688-1693 (1975)
5. Lehane L. Update on histamine fish poisoning. Med. J. Australia 173: 149-152 (2000)
6. Ishizaka T, Ishizaka K, Conard DH, Foese A. A new concept of triggering mechanisms of IgE-mediated histamine release. J. Allergy Clin. Immun. 61: 320-330 (1978)
7. Sgn D. Medications and their use in the treatment of adverse reactions to foods. J. Allergy Clin. Immun. 78: 238-243 (1986)
8. Choi OB, Kim KM, Yoo GS, Park KH. Anti-allergic effects of *Castanea crenata* leaf tea. Korean J. Food Sci. Technol. 30: 468-471 (1998)
9. Schretter AB, Grote M, Vangelista L, Valent P, Sperr WR, Rumpold H, Pastore A, Reichelt R, Valenta R, Spitzauer S. Purification, biochemical, and immunological characterization of a major food allergen: Different immunoglobulin E recognition of the apo- and calcium- bound forms of carp parvalbumin. Gut 46: 661-669 (2000)
10. Lee JW, Yook HS. The changes of allergenic and antigenic properties of egg white albumin (Gal d 1) by gamma irradiation. J. Korean Soc. Food Sci. Nutr. 30: 500-504 (2001)
11. Keun EH, Lee SI, Oh SS. Effect of enzymatic hydrolysis of 7S globulin, a soybean protein, on its allergenicity and identification of its allergenic hydrolyzed fragments using SDS-PAGE. Food Sci. Biotechnol. 15: 128-132 (2006)
12. Shin MY, Han YS, Park HY, Ahn YH, Chung EH, Ahn KM, Lee SI. Cow's milk protein-specific IgE concentrations in two age groups of children with cow's milk allergy. Pediatr. Allergy Respir. Dis. 14: 207-214 (2004)
13. Shimakura K, Tonomura Y, Hamada Y, Nagashima Y, Shiomi K. Allergenicity of crustacean extractives and its reduction by protease digestion. Food Chem. 91: 247-253 (2005)
14. Choi KY, Hong KW. Genomic DNA sequence of mackerel parvalbumin and a PCR test for rapid detection of allergenic mackerel ingredients in food. Food Sci. Biotechnol. 16: 67-70 (2007)
15. Han GD. Heat and high-pressure treatment on *in vitro* digestibility and allergenicity of beef extract. Food Sci. Biotechnol. 15: 523-528 (2006)
16. Kim SH, Kang HR, Kim KM, Kim TB, Kim SS, Chang YS, Kim CW, Bahn JW, Kim YK, Cho SH, Park HS, Lee JM, Min KU, Hong CS, Kim NS, Kim YY. The sensitization rates of food allergens in a Korean population: A multi-center study. J. Asthma Allergy Clin. Immun. 23: 502-514 (2003)
17. Park WP, Kim ZU. The effect of seasonings and salted-fermented fish on *kimchi* fermentation. J. Korean Agric. Chem. Soc. 34: 242-248 (1991)
18. Park YH, Jung LH, Lee SS. Physicochemical characteristics of *tohajeot* added cabbage *kimchi* during fermentation. J. Korean Soc. Food Sci. Nutr. 30: 426-431 (2001)
19. Cha YJ, Lee EH. Studies on the processing of rapid fermented anchovy prepared with low salt contents by adapted microorganism. Bull. Korean Fish. Soc. 22: 363-369 (1989)
20. You BJ, Chang MH. Processing of low salt fermented juice of shellfish with citric acid pretreatment. Korean J. Food Sci. Technol. 24: 541-546 (1992)
21. Lee JW, Kim JH, Yook HS, Kang KO, Lee SY, Hwang HJ, Byun MU. Effects of gamma radiation on the allergenic and antigenic properties of milk proteins. J. Food Protect. 64: 272-276 (2001)
22. Yoon SH, Kim HA, Kim HM, Choi JH, Suh CH, Nahm DH, Kim YK, Min KU, Park HS. Identification of the major allergen in the shrimp (*Metapenaeus Joyneri*): Effects of heating and digestive enzymes. J. Asthma Allergy Clin. Immun. 24: 211-216 (2004)
23. Jeoung BJ, Park KH, Lee HH, Kim KE, Koe SW, Lee KY. Identification and characterization of shrimp allergens in Korea. J. Asthma Allergy Clin. Immun. 17: 278-285 (1997)
24. Daul CB, Morgan JE, Hughes J, Lehrer SB. Provocation-challenge studies in shrimp-sensitive individuals. J. Allergy Clin. Immun. 81: 1180-1186 (1988)
25. Kim SM. Changes in allergenicity of creutacea by fermentation and physical treatment. MS thesis, University of Pukyong National, Busan, Korea (2002)
26. Lee JW, Park JH, Kim SB, Kim CJ, Hyun CK, Shin HK. Application of competitive indirect enzyme-linked immunosorbent assay (Ci-ELISA) for monitoring the degree of frozen denaturation of bovine myosin. Int. J. Food Sci. Tech. 33: 401-410 (1998)
27. Lee JW, Park JH, Kim CJ, Shin HK. Monitoring thermally induced conformational changes in bovine muscle myosin solution by competitive indirect enzyme-linked immunosorbent assay (Ci-ELISA). Int. J. Food Sci. Tech. 33: 411-418 (1998)
28. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685 (1970)
29. Lee JM, Lee HR. Standardization for the preparation of traditional Korean whole cabbage *kimchi* with salted shrimp. Korean J. Diet. Culture 9: 79-85 (1994)
30. Kim JG, Yoon JS. Changes of index microorganisms and lactic acid bacteria of Korean fermented vegetables (*kimchi*) during the ripening and fermentation-part 1. Korean J. Environ. Health 31: 79-85 (2005)
31. Lee IS, Park WS, Koo YJ, Kang KH. Changes in some characteristics of brined Chinese cabbage of fall cultivars during storage. Korean J. Food Sci. Technol. 26: 239-245 (1994)

32. Kang SS, Kim JM, Byun MW. Preservation of *kimchi* by ionizing radiation. Korean J. Food Hyg. 3: 225-232 (1988)
33. Lehrer SB, McCants ML. Reactivity of IgE antibodies with crustacea and oyster allergens: Evidence for common antigenic structures. J. Allergy Clin. Immun. 80: 133-139 (1987)
34. Jin CD, Hong YN. The role of peptide hydrolases on protein degradation in isolated wheat chloroplasts. Korean Biochem. J. 25: 374-380 (1992)
35. Choi SY, Lee MK, Choi KS, Koo YJ, Park WS. Changes of fermentation characteristics and sensory evaluation of *kimchi* on different storage temperature. Korean J. Food Sci. Technol. 30: 644-649 (1998)
36. Min SG, Kim JH, Kim TW, Kim KN. Isolation and identification of proteaes producing bacteria in *kimchi*. Korean J. Food Sci. Technol. 35: 666-670 (2003)
37. Mok CK, Lee JY, Song KT, Kim SY, Lim SB, Woo GJ. Changes in physicochemical properties of salted and fermented shrimp at different salt levels. Korean J. Food Sci. Technol. 32: 187-191 (2000)
38. Oh SH, Heo OS, Bang OK, Chang HC, Shin HS, Kim MR. Microbiological safely of commercial salt-fermented shrimp during storage. Korean J. Food Sci. Technol. 36: 507-513 (2004)
39. Moc CK, Song KT. High hydrostatic pressure sterilization of putrefactive bacteria in salted and fermented shrimp with different salt content. Korean J. Food Sci. Technol. 32: 598-603 (2000)
40. Nam EJ, Oh SW, Jo JH, Kim YM, Yang CB. Purification and characterization of alkaline protease from *saeujeot*, salted and fermented shrimp (*Acetes japonicus*). Korean J. Food Sci. Technol. 30: 82-89 (1998)
41. Kim BM. Changes in the properties of protein during the fermentation of salted shrimp. Korean J. Food Sci. Technol. 20: 883-889 (1988)
42. Bae SW, Choi SY, Jin HS, Kim CW, Lee KE, Kang ES, Park JY, Hong SP, Choi HY, Jung JH, Kim YS, Hong CS. Changes of allergenicity of salted and fermented shrimp. J. Asthma. Allergy Clin. Immun. 23: 44-52 (2003)
43. Barkholt V, Jorgensen PB, Sorensen D, Bahrenscheer J, Haikara A, Laitila A. Protein modification by fermentation: Effect of fermentation on the potential allergenicity of pea. Eur. J. Allergy Clin. Immunol. 53: 106-108 (1998)