Stability and potency of raw and boiled shrimp extracts for skin prick test

Wipada Pariyaprasert,[1] Surapon Piboonpocanun,[2] Orathai Jirapongsananuruk[1] and Nualanong Visitsunthorn[1]

Summary

Background: **The difference of stability between raw and boiled shrimp extracts used in prick tests has never been investigated despite its potential consequences in tests development. The aim of this study was to compare the raw and boiled shrimp extracts of two species; *Macrobrachium rosenbergii* (freshwater shrimp) and *Penaeus monodon* (seawater shrimp) held at 4 ºC for different periods of time for their stability and potency in vivo by using the skin prick test (SPT) method.**

Methods: **Raw and boiled *M. rosenbergii* and *P. monodon* extracts were prepared and stored at 4 ºC for 1, 7, 14 and 30 days. Thirty patients were pricked with raw and boiled shrimp extracts at all storage times, as well as prick to prick skin test (PTP) to fresh raw and boiled shrimps of both species. The mean wheal diameter (MWD) resulting from prick tests for all shrimp extracts was measured and compared.**

Results: **The shrimp extracts of all storage times yielded positive skin test results in the range of 90% - 100%. Raw *P. monodon* extracts induced larger wheals than boiled extracts at all storage times. There was no significant difference of MWD between raw and boiled *M. rosenbergii* extracts on day 1, 7, and 14. Significant correlations between MWD of PTP to fresh shrimps and SPT to all shrimp extracts were observed. All shrimp extracts were sterile at all storage times.**

Conclusions: **Raw and boiled *M. rosenbergii* and *P. monodon* extracts were stable and sterile at 4 ºC for at most 30 days. SPT with these extracts induced more than 10 mm in shrimp allergy patients and the results were comparable with PTP to fresh shrimps. *(Asian Pac J Allergy Immunol 2015;33:136-42)***

Keywords: *skin prick test, prick to prick skin test, raw shrimp extract, boiled shrimp extract, stability of shrimp extract, potency of shrimp extract*

Abbreviation:

MWD	=	Mean wheal diameter
OFC	=	Open food challenge
PTP	=	Prick to prick skin test
SPT	=	Skin prick test

Introduction

Among shellfish, crustaceans such as shrimp, crab, crawfish and lobster are common diets worldwide. Shrimps are the major cause of food hypersensitivity, mainly in adolescents and adults in many countries [1-3] and shrimp allergy is one of the most common seafood allergies among Thai children.[4-5] Immediate allergic reactions to ingested seafood may involve skin, gastrointestinal, respiratory and cardiovascular systems.

Skin prick tests (SPT) are routinely used for initial screening of IgE-mediated food hypersensitivity. The accuracy of skin prick testing for the diagnosis of food allergy depends on several factors, including the character of the allergen extracts,[6] the preservatives,[7] the methods of SPT,[8] and the different SPT measuring methods.[9-10] Ideally, composition and potency of allergen extracts for skin testing should be known. However, most food extracts include seafood are non-standardized mixture.[11] As a consequence, it is important to find a properly known extract for the diagnosis of seafood allergy.

From 1. Division of Allergy and Immunology, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
2. Institute of Molecular Biology and Genetics, Mahidol University, Nakhon Pathom, Thailand
Corresponding author: Nualanong Visitsunthorn
E-mail: nualanongv@yahoo.com
Submitted date: 4/6/2014
Accepted date: 15/10/2014

Macrobrachium rosenbergii (giant fresh water prawn) and *Penaeus monodon* (black tiger shrimp, seawater shrimp) are widely distributed in Southeast Asia and mainly used in preparing Thai food. Tropomyosin has been well classified as a pan-allergen and accounts for most of the allergenic activity in crustacean allergy. However, previous studies have reported species-specific allergenic differences between shrimps.[12-13] Additionally, cooking processes and types of preparation may also affect the allergenicity of seafood proteins.

Our previous work describing specific allergy to *P. monodon* and *M. rosenbergii* in shrimp-allergic children showed that SPT using crude extract and prick to prick skin test (PTP) were useful tools for screening shrimp sensitization before performing oral food challenge (OFC). Both newly prepared Crude extract and PTP gave better results than commercial extracts.[13] Another in vitro study assessed the effects of storage length and conditions on the extracts using specific IgE-allergen profiles and showed that the shrimp extract should be stored at -20 ºC for 4 weeks to prevent the loss of allergens.[14] However, the temperature of standard household refrigerator is only 2-8 ºC and it is legitimate to address the question of whether the extract stored at 4 ºC for one month is still effective for shrimp sensitization. While a previous in vitro study using extract stored for a month at 4 ºC showed fewer antigens as compared to extracts stored at -20 ºC,[14] our in vivo pilot study showed that after storage at 4 ºC for 30 days, SPT with the shrimp extract still gave positive SPT result in shrimp allergic patients. Furthermore, it is important to know the effectiveness of raw and boiled shrimp extracts from different shrimp species in order to select the best allergen extract for the diagnosis of shrimp allergy. Similarly, it is important to compare the results of SPT using raw or boiled shrimp extract over one month period to assess the relevance of preparing extracts in advance and storing them safely for subsequent utilizations.

In this context, the purpose of this study was to compare the raw and boiled shrimp extracts of two shrimp species (*M. rosenbergii* and *P. monodon*) stored for different periods of time at 4 ºC and in vivo by using the skin prick test method.

Methods

Thirty patients, who had a history of positive SPT to commercial shrimp extract (ALK Laboratories, Port Washington, New York), were included in this study. The patients with serious systemic diseases, pregnancy or lactation were excluded. The patients were told to stop using antihistamine and systemic corticosteroids for at least 7 days before the start of the study. All patients were recruited from the Pediatric Allergy Clinic, Siriraj Hospital, Thailand. The study was approved by the Institutional Review Boards, Siriraj Hospital, Mahidol University and informed consents were obtained from the patients or the parents if the patient was less than 18 years old.

Shrimp extract preparations

Shrimp meats *(M. rosenbergii* and *P. monodon*) without shells and heads were frozen. Both raw and boiled (100 ºC, 15 minutes in ion-depleted water) shrimp meats of both species were lyophilized and stored at -20 ºC. Then, they were diluted 1:10 weight/volume in Coca's solution (Siriraj Hospital, Bangkok, Thailand) containing 86 mM NaCl, 42.5 mM phenols, and 29.8 mM $NaHCO_3$. Afterwards, each mixture was kept under continuous magnetic stirring for 1 hour at 4 ºC. Subsequently, the solution was centrifuged at 17,210 g for 30 minutes. Then, the supernatant was sterile filtered.

The shrimp extracts (raw *M. rosenbergii*, boiled *M. rosenbergii*, raw *P. monodon*, and boiled *P. monodon*) were prepared and stored at 4 ºC for 1, 7, 14 and 30 days. At the day of skin testing, fresh raw and boiled shrimp meats of *M. rosenbergii* and *P. monodon* were also prepared for PTP. The shrimp extracts after storage for 7, 14 and 30 days were cultured for sterility testing.

Skin testing

SPTs were performed in all 30 patients with raw and boiled shrimp extracts of both species after different storage times (1, 7, 14 and 30 days), as well as PTP to fresh raw and boiled shrimps. All skin tests were performed with single-use metal lancets. Histamine (10 mg/ml) and 50% glycerosaline solution were used as positive and negative control, respectively. SPTs were applied in a double blinded manner to randomly assigned positions on each patient's back for all extracts. In the standard method, the needle was passed through the drop of extract and pricked the epidermis.[8] PTP was done by pricking fresh raw and boiled shrimp meats and then pricking the skin of the patients. The results were recorded 15 minutes after the test performed by one operator. Each patient was observed for 60 minutes by a physician in a room equipped with emergency material. The diameters of the wheals were outlined with a felt-tipped pen, and

the outlines of the circle were transferred for permanent record storage using tracing paper. The mean wheal diameter (MWD) of each extract was calculated from the sum of the largest measurement across the wheal and the largest wheal measurement perpendicular to the former and divided by two. The SPT result was defined as positive if the MWD was at least 3 mm larger than the negative control.

Statistical analysis

MWD differences between extracts were analyzed using Wilcoxon signed rank test and Paired-samples T test for non-normally and normally distributed data, respectively. Spearman's correlations were used to determine the association between MWD of SPT and PTP. Results were considered statistically significant at a P value of < 0.05.

Results

Thirty patients (13 females and 17 males; mean age ± SD = 13.43 ± 4.08 years) with history of positive SPT to commercial shrimp extract were included into the study. Twenty-eight patients (93%) had a history of immediate hypersensitivity to shrimp. In this group, 20 patients (71.4%) had positive open food challenges (OFC) to shrimp while three patients (10.7%) had negative OFC. The remaining seven patients were not evaluated. Two patients from the unevaluated group (7.1% of the cases that had a history of immediate hypersensitivity to shrimp) were asymptomatic after accidental shrimp ingestion.

The shrimp extracts of both species, stored for 1, 7, 14 and 30 days, were all sterile. MWD of shrimp extracts are presented in Table 1. When stored at 4 ºC, MWD of raw and boiled *M. rosenbergii* at different storage times (Day 1, 7, 14 and 30) were not statistically significant but there was a statistically significant difference among MWDs of raw and boiled *P. monodon* extract at different storage times. All shrimp extracts at all storage times yielded 90% to 100% of positive skin tests with MWD larger than 10 mm. Comparisons of mean wheal diameter of PTP to fresh *P. monodon* and SPT to raw and boiled *P. monodon* extracts at

Table 1. Mean wheal diameter of skin prick test of raw and boiled *M. rosenbergii* and *P. monodon* extracts at different storage times.

1A. Raw and boiled *M. rosenbergii* extracts.

Type of shrimp	Wheal diameter (mm) at different storage times (mean ± SD)				*p*-value*
	Day 1	Day 7	Day 14	Day 30	
Raw *M. rosenbergii* (median)	14.67 ±1.84 (11.75)	14.80 ±1.19 (14.50)	14.23 ±1.46 (13.25)	14.52 ±1.24 (13.50)	0.419
Boiled *M. rosenbergii* (median)	13.17 ±1.17 (12.75)	13.18 ±1.05 (13.50)	12.07 ±0.94 (12.75)	12.10 ±1.19 (12.00)	0.373
p-value**	0.21	0.16	0.081	0.036	

1B. Raw and boiled *P. monodon* extracts.

Type of shrimp	Wheal diameter (mm) at different storage times (mean ± SD)				*p*-value*
	Day 1	Day 7	Day 14	Day 30	
Raw *P. monodon* (median)	15.82 ±1.88 (14.25)	15.00 ±1.46 (14.00)	14.70 ±1.36 (14.00)	12.13 ±1.05 (11.50)	0.03
Boiled *P. monodon* (median)	11.62 ±1.12 (11.00)	12.03 ±1.28 (12.50)	10.07 ±0.97 (9.75)	10.90 ±0.96 (10.75)	0.051
p-value**	0.026	0.062	< 0.0001	0.051	

p-values* Compare mean wheal diameter of each shrimp extract among all storage times (1, 7, 14 and 30 days)

p-values** Compare mean wheal diameter between raw and boiled shrimp extracts within each storage time

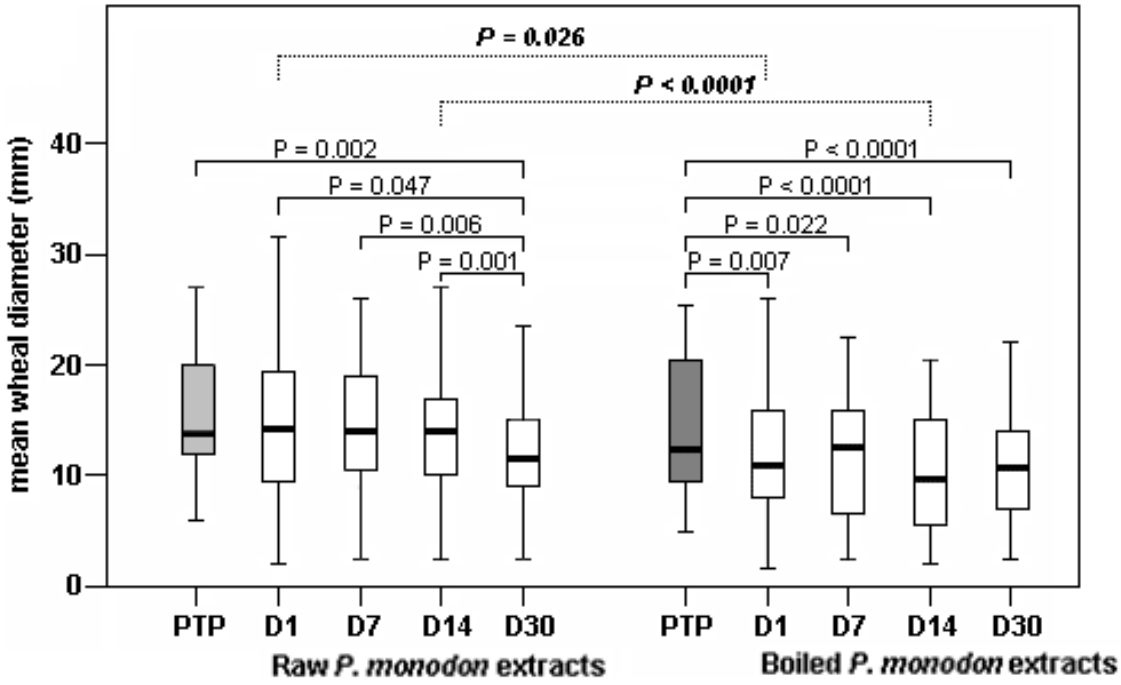

Figure 1. Comparison between mean wheal diameter of prick to prick skin test (PTP) to fresh *P. monodon* and skin prick test to raw and boiled *P. monodon* extracts at all storage times (day 1, 7, 14 and 30).
(·····) *p*-values when compare between raw and boiled shrimp extracts
(——) *p*-values when compare between PTP and SPT at all storage times

all storage times are shown in Figure 1. The MWD of raw *P. monodon* extract at 30 days of storage was significantly smaller than those of PTP and at 1, 7 and 14 days of storage (P = 0.002, 0.047, 0.006, and 0.001 respectively). The MWD of PTP of boiled *P. monodon* extract was significantly larger than those of the boiled extracts stored at 1, 7, 14 and 30 days (P = 0.007, 0.022, <0.0001 and < 0.0001 respectively). The significant larger MWD of raw *P. monodon* extract compared to boiled extract was found at Day1 and 14 (P = 0.026 and P < 0.0001 respectively).

There was no significant difference of MWD between raw and boiled *M. rosenbergii* extracts at 1, 7 and 14 days of storage. The MWD of raw *M. rosenbergii* extract after storage for 30 days was significantly larger than that of the boiled extract (P = 0.036) as shown in Figure 2. There was no statistically significant difference between MWD of PTP to fresh shrimps and SPT to both raw and boiled shrimp extracts of *M. rosenbergii*.

There were moderate correlations between MWD of PTP to fresh shrimps and SPT to all shrimp extracts of both *M. rosenbergii* and *P. monodon* at 1 day of storage (r = 0.59 – 0.75, P < 0.005) as shown in Figure 3. Similarly, MWD of all shrimp extracts stored for 7, 14 and 30 days had a significant correlation with PTP to fresh shrimps as shown in Table 2.

Discussion

Shrimp is often recognized as the cause of adverse food reactions in hypersensitive individuals worldwide. Tropomyosin is a major allergen in many shellfish, especially crustaceans and mollusks. Lehrer et al.[15] demonstrated that tropomyosin was a 36-kDa protein to which more than 80% of shrimp-allergic subjects reacted. They also suggested that this protein was present in both cooked and uncooked samples of two different species of shrimps, *Penaeus aztecus* and *Penaeus setiterus*.

Our previous work showed that both newly prepared crude extract and PTP gave better result than commercial extract.[13] Another in vitro study showed that the shrimp extract from raw shrimp should be stored at -20 ^{o} C for 4 weeks to prevent the loss of allergens but the boiled shrimp extract could be stored at 4 ^{o}C for a month without significant reduction in low molecular weight allergens

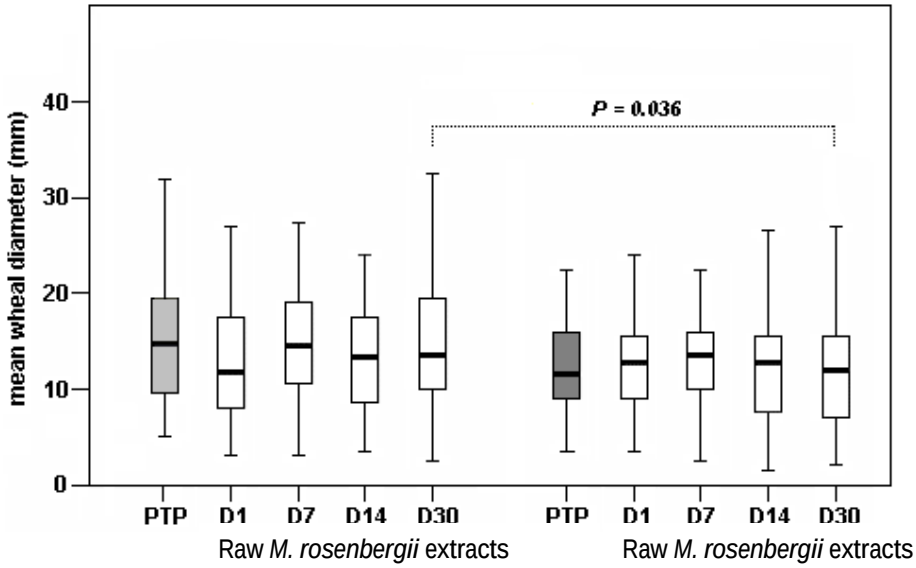

Figure 2. Comparison between mean wheal diameter of prick to prick skin test (PTP) to fresh *M. rosenbergii* and skin prick test to raw and boiled *M. rosenbergii* extracts at all storage times (day 1, 7, 14 and 30).
(·····) *p*-values when compare between raw and boiled shrimp extracts

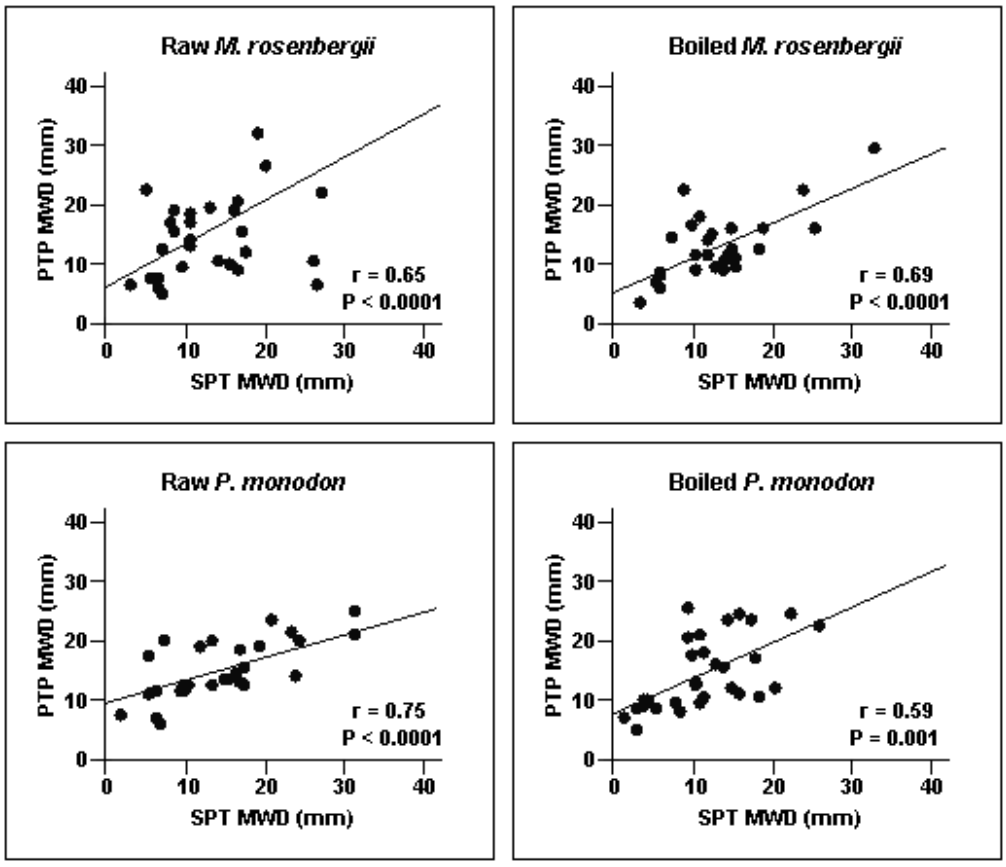

Figure 3. Correlations between mean wheal diameter (MWD) of skin prick test (SPT) to raw and boiled shrimp extracts of both species at 1-day storage and prick to prick skin test (PTP) to fresh raw and boiled shrimps.

Table 2. Correlations between mean wheal diameter of skin prick test to raw and boiled shrimp extracts at different storage times and prick to prick skin test to fresh shrimps in parallel groups.

	Correlation Coefficient (r)			
	Day1	Day7	Day14	Day30
Raw *M. rosenbergii*	0.65 *P* <0.0001	0.62 *P*<0.0001	0.86 *P* <0.0001	0.50 *P* =0.005
Boiled *M. rosenbergii*	0.69 *P* <0.0001	0.43 *P* =0.018	0.40 *P* =0.029	0.65 *P* <0.0001
Raw *P. monodon*	0.75 *P* <0.0001	0.58 *P* =0.001	0.66 *P* <0.0001	0.59 *P* =0.001
Boiled *P. monodon*	0.59 *P* =0.001	0.62 *P* <0.0001	0.64 *P* <0.0001	0.66 *P* <0.0001

(<50kDa) which were major shrimp allergen components. [14]

This study showed that the local shrimp extracts from both boiled and raw shrimp, stored in 4 ºC for at least a month, were still effective in detecting shrimp allergy by SPT. However, the study by Carnes et al. showed that the use of boiled shrimp extracts seemed to be more effective than raw extracts in the diagnosis of seafood allergy.[16] The authors of this study found that boiled prawn extracts (*Penaeus* sp) induced greater percentage of positive skin test reactions than raw extracts. Moreover, the mean wheal sizes of boiled shrimp extracts were significantly greater than that of raw extracts.[16]

These findings contrast with ours as MWD of boiled *P. monodon* extracts was significantly smaller than that of raw extracts. The discrepancy between our studies may have resulted from the implementation of different methods of shrimp extracts preparation. Interestingly, there was no significant difference of MWD between raw and boiled extracts of *M. rosenbergii* and we postulated that the cooking process affected the antigenicity of *P. monodon* extracts, but not *M. rosenbergii*.

Species of the shrimp genera *Penaeus* and *Metapenaeus* were the most thoroughly studied crustacean allergens[17-18] but there are still a very limited number of studies regarding *M. rosenbergii* or freshwater shrimps. The differences of hypersensitivity reactions related to various shrimp species may be explained by the possibility of a species-specific type of allergenicity, and patients IgE antibodies against shrimp proteins may be specific for members of various shrimp families.[19] Additionally, in this study, thermal processing provided different effects on species-specific shrimp extracts. The potentially multiplicative effects of species-specific allergenicity in freshwater and saltwater schrimps and thermal processing are important to investigate further in order to better understand hypersensitivity reactions and develop more accurate allergy screening tests.

In this study, we documented the deterioration of the allergen extracts over time. The potency of the extracts may decline from several factors, such as dilution, storage at improper temperature, or the mixture with other allergen extracts.[20] The stability of allergen preparations can be estimated with either in vivo or in vitro methods,[21] the difference in stability between raw and boiled shrimp extracts has never been reported. We determined the stability of various shrimp extracts during 30 days of storage by using skin test reactivity. Clinically, all shrimp extracts at all storage times yielded 90% to 100% of positive skin tests with MWD of larger than 10 mm. Our results therefore suggest that raw and boiled shrimp extracts of *M. rosenbergii* and *P. monodon* are stable enough over time to be appropriately used as skin test extracts for at most 30 days. Further studies are required to evaluate the stability for longer periods.

In this study, we also demonstrated moderate correlation between SPT to shrimp extracts and PTP to fresh shrimps. Therefore, we suggest that both raw and boiled shrimps, which were lyophilized before dilution and extraction, may be appropriate for preparing SPT extracts for the diagnosis of seafood allergy. The lyophilized shrimps, when compared with fresh shrimps for PTP, provide greater advantage in allowing longer storage, easier usage, and reduction of variation between lots.

Conclusions

Raw and boiled *M. rosenbergii* and *P. monodon* extracts were stable and sterile at 4 ºC for at most 30 days. SPT with these extracts induced a MWD larger than 10 mm in shrimp allergy patients and the results were comparable with PTP to fresh shrimps.

Acknowledgments

This study was supported by Mahidol University and the National Research University grant from Commission on Higher Education (CHE) through the Center for Biopharmaceutical Development and Innovative Therapy, Mahidol University, Thailand.

References

1. Sichere SH, Munoz-Furlong A, Sampson HA. Prevalence of seafood allergy in the United States determined by a random telephone survey. J Allergy Clin Immunol. 2004;114:159-65.
2. Crespo JF, Rodriguez J. Food allergy in adulthood. Allergy. 2003; 58:98-113.
3. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: A meta-analysis. J Allergy Clin Immunol. 2007;120:638-46.
4. Chaidaroon D, Visitsunthorn N, Vichyanond P. Seafood allergy: Clinical manifestation in Thai patient who was referred to referral allergy center, Siriraj Hospital. J Allergy Clin Immunol. 2003;111:s106.
5. Jirapongsananuruk O, Bunsawansong W, Piyaphanee N, Visitsunthorn N, Thongngarm T, Vichyanond P. Features of patients with anaphylaxis admitted to a university hospital. Ann Allergy Asthma Immunol. 2007;98:157-62.
6. Rance F, Juchet A, Bremont F, Dutau G. Correlations between skin prick tests using commercial extracts and fresh foods, specific IgE, and food challenges. Allergy. 1997;52:1031-5.
7. Morell F, Codina R, Rodrigo MJ. Increased positivity of skin test and allergenic stability of glycerinated soybean hull extracts. Clin Exp Allergy. 1999;29:388-93.
8. Zawodniak A, Kupczyk M, Gorski P, Kuna P. Comparison of standard and modified SPT method. Allergy. 2003;58:257-9.
9. Ueno H, Yoshioka K, Matsumoto T. Usefulness of the skin index in predicting the outcome of oral challenges in children. J Investig Allergol Clin Immunol. 2007;17:207-10.
10. Eigenmann PA, Sampson HA. Interpreting skin prick tests in the evaluation of food allergy in children. Pediatr Allergy Immunol. 1998;9:186-91.
11. Bernstein L, Li JT, Bernstein DI, Hamilton R, Spector SL, Tan R, et al. Allergy diagnostic testing: an updated practice parameter. Ann Allergy Asthma Immunol. 2008;100:S1-148.
12. Morgan JE, O'Neil CE, Daul CB, Lehrer SB. Species-specific shrimp allergens: RAST and RAST-inhibition studies. J Allergy Clin Immunol. 1989;83:1112-7.
13. Jirapongsananuruk O, Sripramong C, Pacharn P, Udompunturak S, Chinratanapisit S, Piboonpocanun S, et al. Specific allergy to Penaeus monodon (seawater shrimp) or Macrobrachium rosenbergii (freshwater shrimp) in shrimp-allergic children. Clin Exp Allergy. 2008;38:1038-47.
14. Piboonpocanun S, Boonchoo S, Pariyaprasert W, Visitsunthorn N, Jirapongsananuruk O. Determination of storage conditions for shrimp extracts: analysis of specific IgE-allergen profiles. Asian Pac J Allergy Clin Immunol. 2010;28:52.
15. Lehrer SB, Ayuso R, Reese G. Seafood allergy and allergens: a review. Mar Biotechnol. 2003;5:339-48.
16. Carnes J, Ferrer A, Huertas AJ, Andreu C, Larramendi CH, Caldas EF. The use of raw or boiled crustacean extracts for the diagnosis of seafood allergic individuals. Ann Allergy Asthma Immunol. 2007;98:349-54.
17. Reese G, Ayuso R, Lehrer SB. Tropomyosin: an invertebrate pan-allergen. Int Arch Allergy Immunol. 1999;119:247-58.
18. Reese G, Schicktanz S, Lauer I, Randow S, Luttkopf D, Vogel L, et al. Structural, immunological and functional properties of natural recombinant Pen a 1, the major allergen of brown shrimp, Penaeus aztecus. Clin Exp Allergy. 2006;36:517-24.
19. Samson KTR, Chen FH, Miura K. IgE binding to raw and boiled shrimp proteins in atopic and nonatopic patients with adverse reactions to shrimp. Int Arch Allergy Immunol. 2004;133:225-32.
20. Nelson HS, Ikle D, Buchmeier A. Studies of allergen extract stability: the effects of dilution and mixing. J Allergy Clin Immunol. 1996;98:382-8.
21. Niemeijer NR, Kauffman HF, Hove WV, Dubois AE, Monchy JGR. Effect of dilution, temperature, and preservatives on the long-term stability of standardized inhalant allergen extracts. Ann Allergy Asthma Immunol. 1996;76:535-40.