Protein Composition Changes in Manufactured Penaeus aztecus Shrimp Powder

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Introduction

Shellfish (crustacaen) allergy is the most common food allergy in adults and the third most common in children in the United States, causing severe reactions such as anaphylaxis[1,2]. However, shrimp allergy is not well understood or characterized for different shrimp species. Shrimp proteins are highly allergenic and the protein composition can be affected by methods of manufacturing. Understanding the proteins in shrimp powder is important due to use in skin tests and food challenges, the gold standard of the diagnosis of food allergy. Among the shrimp-identified allergens, the heat-stable muscle protein Tropomyosin is a major allergen across species and is highly conserved among invertebrates[3,4]. Other, less heat-stable, shrimp allergen proteins identified in multiple species are Arginine kinase, Myosin light chain and Sarcoplasmic calcium-binding protein, involved in energy and muscle activities. The Good Manufacturing Practice (GMP) Manufacturing Facility staff at the Sean N. Parker Center for Allergy and Asthma Research (SNP) manufactured brown shrimp, (Farfante) Penaeus aztecus, powder and compared it to shrimp skin prick test extract material, and to raw and cooked shrimp.

Methodology

Samples of brown shrimp, (Farfante) Penaeus aztecus, were processed under the following conditions as per Table 1: 1) desiccated only, 2) desiccated followed by heat for 15 minutes at 350°F as an end step to minimize microbial contaminants, 3) microwaved for 20 seconds, and 4) raw. Manufactured flours were tested for microbial contaminants. The samples were compared to flour used in shrimp skin prick test extract (Stallergenes Greer®, North Carolina, USA). Phosphate-buffered saline (PBS) extract of flour used in shrimp skin test prick extract was also compared to skin prick test extract.

Table 1. Sample processing

#	Sample	Preparation condition
1	Shrimp flour	Desiccation only
2	Shrimp flour	Desiccation followed by 15 minutes of heating (350°F)
3	Shrimp	Microwave (20 seconds)
4	Shrimp	Raw

All samples were prepared in sodium dodecyl sulfate (SDS) buffer and analyzed by reducing polyacrylamide gel electrophoresis (SDS-PAGE) using standard procedures followed by Coomassie Blue staining and densitometry readings (ImageLab, Bio-Rad).

Results

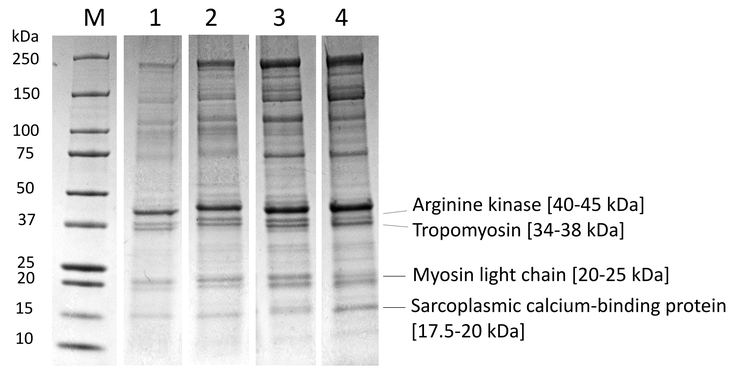

Figure 1. (A) SDS-PAGE analysis of shrimp preparations and skin prick test extract.

1. **Shrimp flour:** Desiccation and heating end-step
2. **Shrimp flour**: Desiccation only
3. **Shrimp**: Raw
4. **Shrimp flour** used for skin prick test extract

Arginine kinase (40-45 kDa), Tropomyosin (34-38 kDa), Myosin light chain (20-25 kDa) and Sarcoplasmic calcium-binding protein (17.5-20 kDa) bands differ in intensity (i.e. concentration) depending on the preparation method. Removing the heat as an end step preserves all proteins and is more comparable to the flour used in skin prick test extract (which is manufactured without cooking/heating).

Table 2. Detected protein levels of shrimp allergens as ratio to flour used for skin prick test extract.

Shrimp allergen	Desiccation + heating	Desiccation - heating	Flour SPT extract	Shrimp raw
Arginine kinase	0.65	0.90	1	0.78
Tropomyosin	0.75	1.01	1	1.30
Myosin light chain	0.53	0.73	1	0.83
Sarcoplasmic calcium-binding protein	0.44	0.66	1	1.20

Densitometry analysis of probable shrimp allergen proteins (Table 2) confirmed that Tropomyosin is a heat-stable protein with no change compared to skin prick test flour for the desiccation only flour preparation. The preparation which included a heating end-step reduced Tropomyosin band intensity by 25% compared to skin prick test flour. Other shrimp allergen proteins were reduced by between 10 and 34% for desiccation only preparation, and between 25 and 56% for desiccation with heating end-step. Arginine kinase (-10 and -35%, respectively) appeared more heat-stable than Myosin light chain (-27 and -47%), and least heat-stable was Sarcoplasmic calcium-binding protein (-34 and -56%).

Results (contd.)

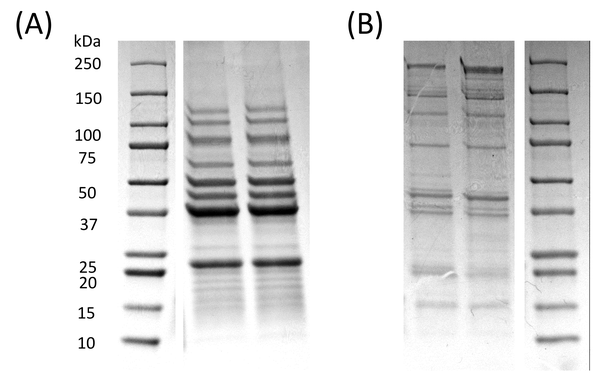

Figure 2. (A) SDS-PAGE analysis of PBS extract of flour used in skin prick test extract (left) and skin prick test extract (right).
(B) SDS-PAGE analysis of microwaved (left) and raw (right) shrimp.

PBS extract of the flour used skin prick test and the skin prick test extract resulted in similar gel profiles though distinct from SDS-treated samples (Figure 2A). Differences in water solubility for the various proteins may account for these differences. Comparison between microwaved and raw shrimp revealed no visible differences (Figure 2B).

Limitations

Skin prick test extract preparations differs from the flour preparations used in the study and therefore the two cannot be directly compared. However, flour-to-flour comparisons can be directly made.

Conclusion

Cooking methods alter protein profiles in brown shrimp. Skin prick test extract flour most closely mimics raw shrimp; however, if shrimp powders are used for food challenges, we recommend performing studies to ensure all proteins are represented to avoid false negative food challenges.

References

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2. Wang HT, Warren CM, Gupta RS, Davis CM. Prevalence and Characteristics of Shellfish Allergy in the Pediatric Population of the United States. Allergy Clin Immunol Pract. 2020 Jan 7. pii: S2213-2198(19)31061-X.
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Funding

This work was supported through the Sean N Parker Center for Allergy and Asthma Research at Stanford University and NIH NAID U01 RFA-AI-16-027.