

DETECTION OF CRUSTACEANS WITH AN ANTISERUM TO TROPOMYOSIN

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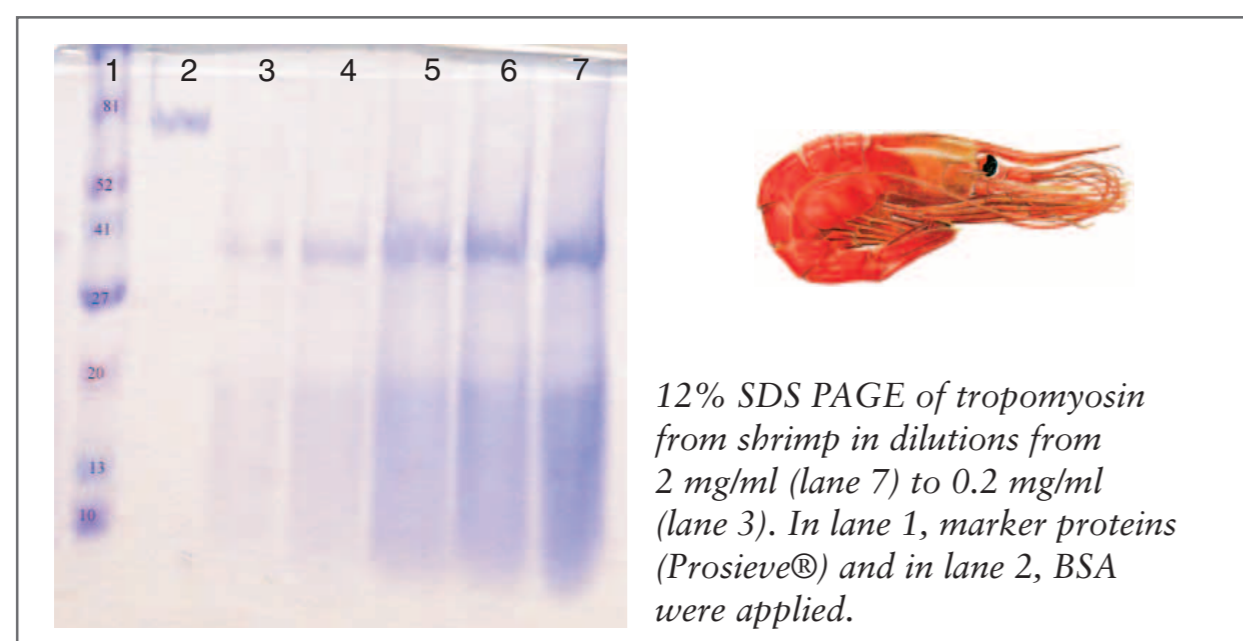
Introduction

Crustaceans are the fourth most common cause of severe food reactions in Europe and accounted for 50 out of 613 reported severe food reactions (SCOOP Nutr Report 2/98).

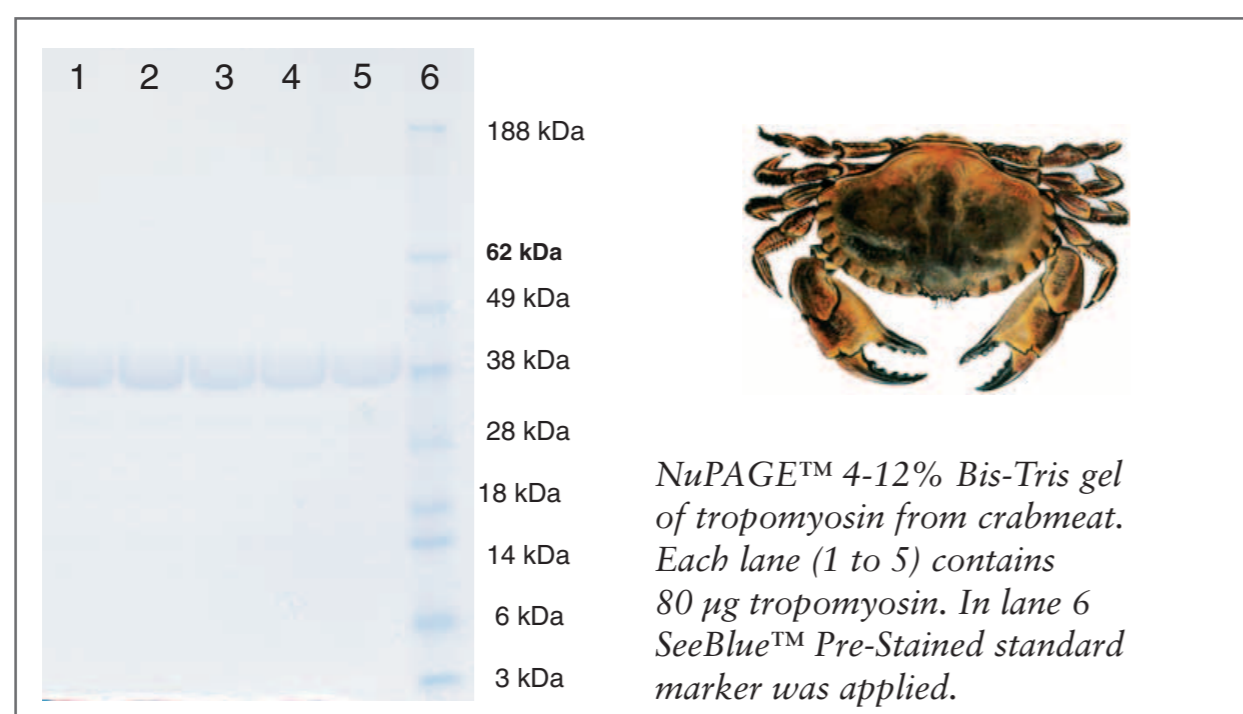
Crustaceans and products thereof are also recognised by Codex Alimentarius and EU among those allergens that should always be declared. The access to methods for the detection of crustaceans in food is important to protect the allergic consumer. In addition, the same methods can be used to detect fraud in surimi products since higher price is attributed to products containing crabmeat compared to those without.

Tropomyosin isolation

The major allergen in crustaceans, tropomyosin, was isolated from shrimps (*Pandalus borealis*) by incubation in Tris/glycine buffer, 1 mM DTE, pH 8.7 at 45°C overnight and centrifuged at 4°C. The supernatant was then precipitated twice with ammonium sulphate, resuspended in Tris buffer, 1 mM DTE, pH 8.0, ultra-filtered, dialysed against water, freeze-dried and finally dissolved in PBS. The protein content was adjusted to 2 mg/ml.



In addition, tropomyosin was isolated from crab (*Cancer pagurus*) with a similar technique.



Conclusion

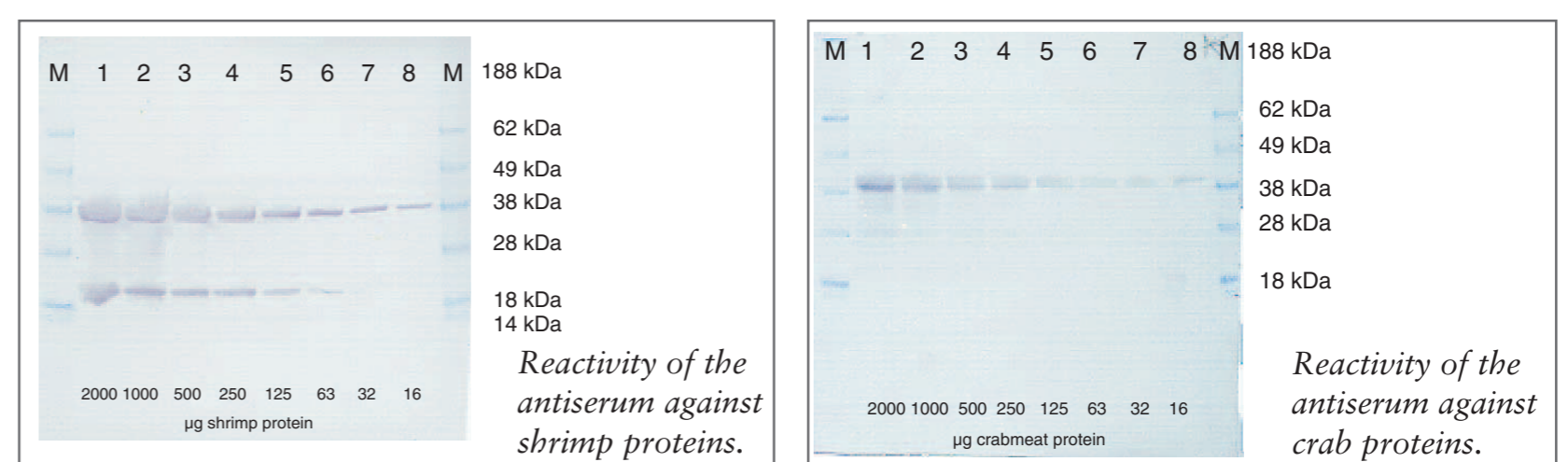
By using antibodies to tropomyosin it is possible to detect the presence of low levels of shrimp and crab in food. Even in highly processed samples like surimi it is possible to quantify the amount of crabmeat.

Characterization of the antiserum to tropomyosin

Antiserum against shrimp tropomyosin was raised in a rabbit. The antiserum was characterized by immunoblotting EIA and RIE.

Immunoblotting

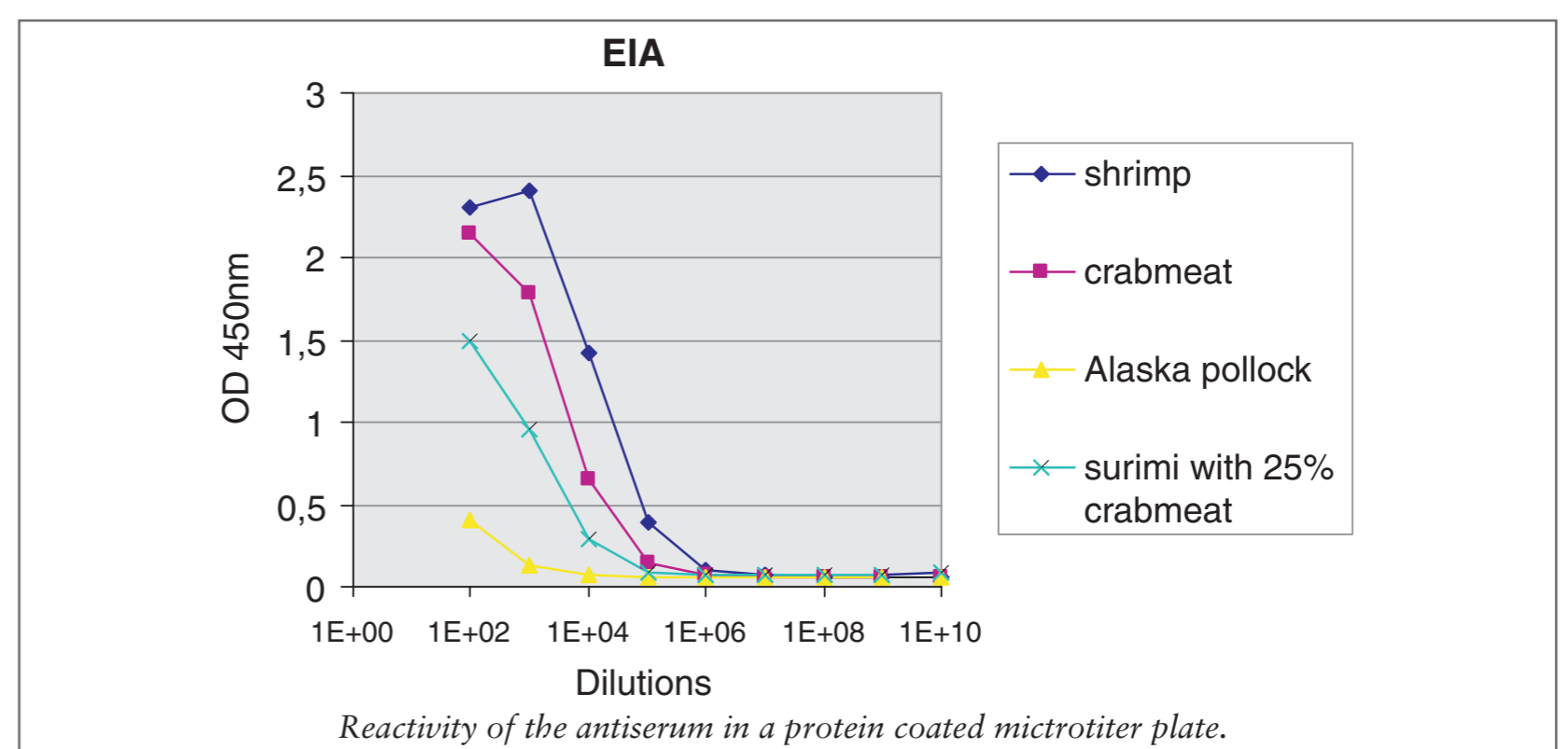
2 g of shrimp or crabmeat was extracted in buffer in a total volume of 10 ml. Two-fold dilutions of each extract were applied to two gels (NuPAGE™, 4-12% Bis-Tris gel). After separation, the proteins were blotted onto nitrocellulose sheets. The sheets were first protein-stained (Ponceau) and then immunostained with rabbit anti tropomyosin, followed by goat anti rabbit, PAP complex and substrate for the enzyme. On each gel 2000 µg protein was applied in lane 1 and dilutions down to 16 µg meat of shrimp or crab, respectively, in lane 8. In lanes M, markers (SeeBlue™ Pre-Stained standard) were applied.



Immunoblotting revealed a dominant reactivity of the antiserum towards a protein in shrimp of approximately 38 kDa. Reactivity was also recognised towards a protein of 20 kDa in shrimp. In crab (*Cancer pagurus*) only one single protein band of approximately 40 kDa was detected by the antiserum.

Enzyme Immuno Assay – EIA

The antiserum was further tested in an EIA. A microtiter plate was coated with 0.5 µg protein per ml of extracts of shrimp, crab, Alaska pollock and a surimi product, containing 25% crabmeat. The antiserum gave a clear signal to all extracts except for the fish species. At a dilution of 1:10 000, the optical density from Alaska pollock was less than 0.1 while the surimi gave an OD of 0.28, the crab 0.65 and the shrimp 1.4.



Rocket immunoelectrophoresis assay – RIE assay

5 g of meat from crab, shrimp, Alaska pollock and a surimi product containing 25% crabmeat were extracted in buffer in a total volume of 10 ml. The protein concentrations of the extracts were determined by BioRad protein assay kit. Samples were diluted 1:200. Extracts of crab and shrimp were further diluted two-folds before being applied in the wells of RIE plates. 40 µl of the antiserum to tropomyosin was added to 2 ml agarose prior to the moulding of the gels.

Shrimp and crab were detected down to a protein concentration of 5 ppm. The assay detected 20-40 ppm crab in a surimi product. No precipitin line was seen with Alaska pollock.

