DEVELOPMENT OF NEW ANALYTICAL METHOD FOR THE DETECTION OF ALLERGENS IN SEA FOOD

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RESUME

Cet article présente une méthode pour la détection d'un allergène majeur qui est la tropomyosine chez certaines espèces tunisiennes de fruits de mer. La discrimination de cet allergène a été effectuée par électrophorèse et par chromatographie d'exclusion. La validation de cette méthode sur des échantillons réels montre la présence de la tropomyosine dans des espèces étudiées.

ABSTRACT

This paper presents a method for detecting the major allergen which is the tropomyosin in some species of Tunisian seafood. Discrimination of this allergen was performed by electrophoresis and by exclusion chromatography. Validation on real samples shows the presence of tropomyosin in the studied species.

INTRODUCTION

The benefits of seafood for human health are very important and their consumption is increasing. The prevalence of the consumption of marine products is due to their nutritional value reflected by their high fat content, and more specifically in fatty acids (Connor, 2000). Currently, seafood such as shrimp, clams and prawns are among the food products which are marketed better internationally and this situation will continue until 2030 (FAO).

However, these products can potentially cause severe immune reactions, up to the fatal anaphylaxis (Yunginger et al., 1988). Allergic reactions caused by consumption of fish and seafood are due to the presence of certain proteins in their muscles (Lehrer et al 2003).

Allergy to shellfish is essentially due to two proteins formally identified as tropomyosin and arginase kinase (Leung and Chu 2001).

There are several bio- analytical methods (immunoassay, PCR) for the detection of allergens, each offering advantages and disadvantages, therefore the technical identification and quantification continue to grow (Heick., et al 2001).

Recently the use of High performance liquid chromatography coupled with mass spectrometry (LC / MS / MS) has emerged as a new tool to detect allergens and established itself as the only technology that can provide confirmation of the presence of several types of allergens in a single analysis.

In this context, fits our principal objective of developing reliable and rapid methods to identify and quantify allergens caused by eating seafood.

MATERIELS and METHODS

Chemicals and Sample

All chemicals reagents were of pure grade. Sodium dodecylsulfate (SDS), Nacl, phosphate buffer and standard tropomyosin buffer were from SIGMA.

Shrimp, prawn, oysters and mussel were sampled from the north of Tunisia only crab was sampled from the south of Tunisia. Muscle samples were either immediately used or stored at 80°C.

Protein extraction

Extracts were prepared from raw and boiled muscle after modification of Kamath et al, 2013 method. About 5 g of the muscle mass was homogenised in 15 ml of phosphate buffered saline (PBS) with 3% Nacl for 10 min, using an Ultra turrax blender. This slurry was then centrifuged at 12000 rpm for 15 min, the supernatant was filtered by membrane filter 0, 45 µm and stored at -20°C prior further use.

For preparation of heated protein extracts, a more natural way of heat treatment was used, instead of just heating the raw extract, to mimic the way consumers are usually exposed to food allergens. The complete specimen, with its outer shell, was heated in distilled water at 100 °C for 20 min.

SDS-PAGE analysis

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed under reducing conditions according to the method of Laemmli (1970).

Samples were separated in 12% polyacrylamide gels with 5% stacking gel and the gels were stained for protein with Coomassie Brilliant Blue R-250.

Exclusion Chromatography analysis

Size-exclusion chromatography (SEC) is a chromatographic method in which molecules in solution are separated by their size, and in some cases molecular weight. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers.

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RESULTS

SDS-PAGE profile

The purified tropomyosin extract was profiled by SDS-PAGE as illustrated in (Figure 1). In all of the samples shrimp, oyster and mussel band of 36 kDa is presumed to be the major allergen present in different species

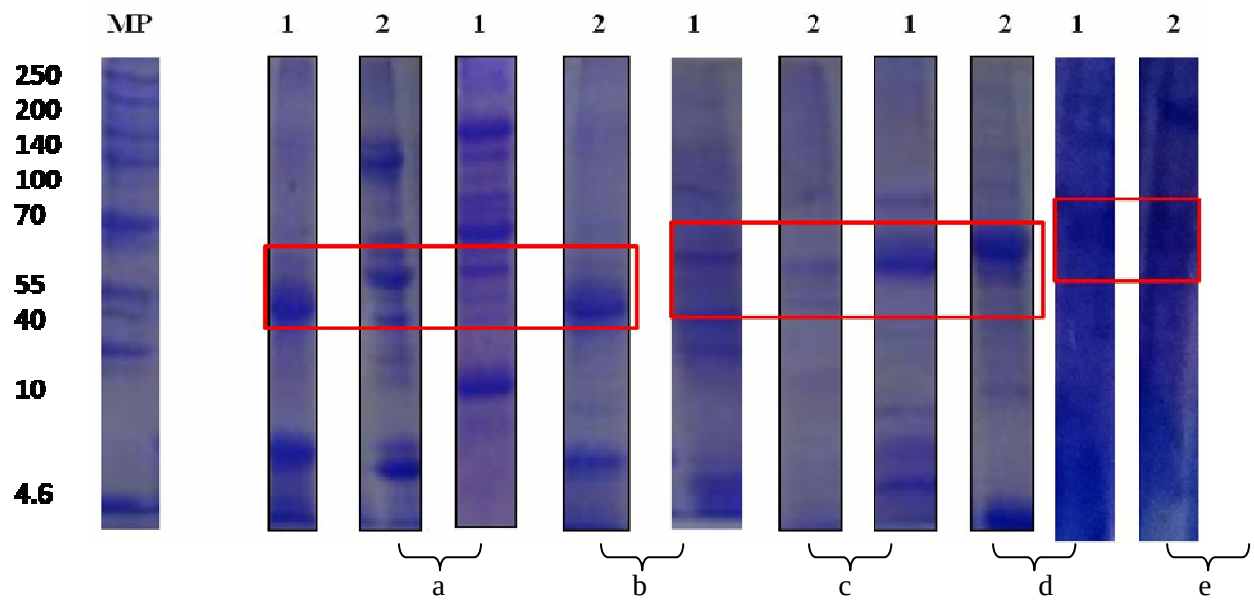

Figure 1 : electrophoresis profiling of major allergen protein in the crude (1) and heat (2) muscle of (a) shrimp, (b) prawn, (c) mussel, oyster (d) and crab (e)

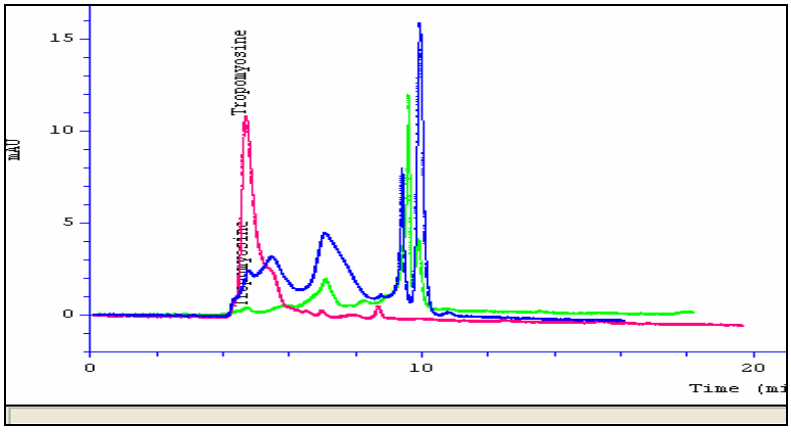

Figure 2 : chromatogram of Shrimp (green line) and prawn (blue line) obtained with exclusion chromatography

Exclusion chromatography

For the results of chromtograhphy there are confirmations of the results obtained with electrophoresis. The major allergen observed is tropomyosin. This result observed in the fig. 2 and fig.3

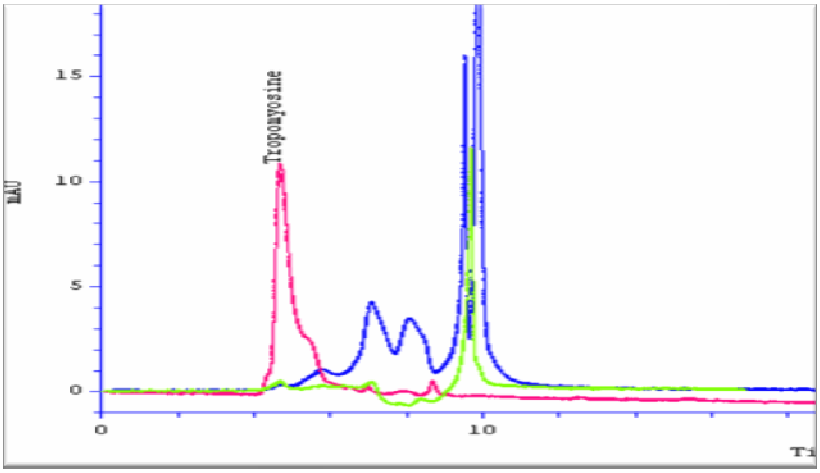

Figure 3 : chromatogram of oyster (green line) and mussel (blue line) obtained with exclusion chromatography

DISCUSSION

Consumption of seafood can produce allergic symptoms in susceptible individuals and crustacean allergies are one of the most frequently reported causes of allergic reactions. our results obtained showed the presence of tropomyosin in shrimp muscle, these results are similar to results performed on shrimp *Parapenaeus fissurus* by yee-lin et al, 1993[7] and black tiger prawn *Penaeus monodon* by Abdelrahman et al, 2010.
the same results were found in the study conducted on crab by Liang et al, 2008, other papers have studied them as oysters and mussels Ai et al., 2009 have shown the same results as ours with a major allergen and tropomyosin and arginine kinase.

CONCLUSION

In this paper we show that the muscle of our sample contain the tropomyosin allergen, but we need to quantify this allergen with a new analytical methods based in the microplate.

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