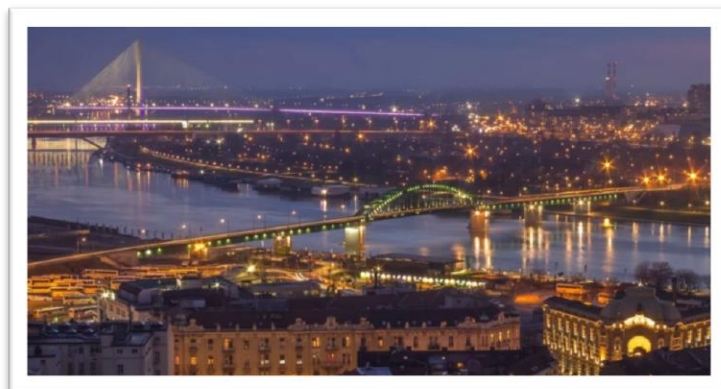




# FoodEnTwin Symposium: Novel analytical approaches in food and environmental sciences Book of Abstracts



June 16-18, 2021  
Belgrade, Serbia



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## DETECTION AND CHARACTERIZATION OF TROPOMYOSIN FROM ANADARA SEASHELLS USING AN IMMUNOPROTEOMICS APPROACH

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**Introduction:** Shellfish allergy is one of the most common food allergies with a prevalence of 0.5% - 2.5% in the general population. The most common allergen present in shellfish is tropomyosin. Detection of tropomyosin in seashells is a challenge because there are no specific antibodies for seashells' tropomyosin. Our aim was to verify the presence of tropomyosin in *Anadara* seashells using an immunoproteomic approach and to investigate the level of cross-reactivity with shrimps.

**Methods and Results:** Proteins from lyophilized seashells *Tegillarca granosa* (TG) and *Anadara broughtonii* (AB) were extracted in: RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 0,1% SDS, 150 mM NaCl, 50 mM Tris-HCl, 1mM EDTA) and Rehydration buffer (7M urea, 2M thiourea, 2% CHAPS and 10mM DTT). Protein concentration of extracts was determined by Bradford assay and SDS-PAGE. The presence of tropomyosin has been supported by commercial tropomyosin standard in 1D SDS-PAGE. With 1D immunoblot, it was possible to confirm the reactivity of seashells' tropomyosin to rabbit anti-shrimp tropomyosin polyclonal antibodies, confirming its presence. Tropomyosin's presence was also validated with 1D immunoblot using monoclonal antibodies. 2D electrophoresis showed that most of samples' proteins are in acidic pI range with prevalence of spots in the range 35-50kDa, and, by comparing spots to 2D immunoblot with polyclonal antibodies, it is possible to confirm tropomyosin's presence in *Anadara* seashells.

**Conclusions:** We found that tropomyosin is present in both blood clam species. Both monoclonal and polyclonal antibodies raised against shrimp tropomyosin can detect seashells tropomyosin by immunoblot pointing to a potential antibodies cross-reactivity of allergic subjects to shrimps and seashells.

**Keywords:** Immunoproteomic approach, shellfish allergy, tropomyosin, cross-reactivity, shrimp

**Acknowledgements:** This research work was funded the Ministry of Education, Science and Technological Development of Republic of Serbia, through contract number: 451-03-9/2021-14/200168; Belgian Special Research Fund BOF StG No. 01N01718; Serbian Academy of Sciences and Arts GA No. F-26, and the European Commission, under the Horizon2020, FoodEnTwin project, GA No.810752.