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Immuno-PCR for crustacean tropomyosin quantification

Mirjana Radomirović[1*], Nikola Gligorijević[2], Dragana Stanić-Vučinić[1], Andreja Rajković[3,4], Tanja Ćirković Veličković[1,3,4,5]

[1]Center of Excellence for Molecular Food Sciences and Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia
[2]Center for Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade
[3]Ghent University Global Campus, Yeonsu-gu, Incheon, South Korea
[4]Faculty of Bioscience Engineering, Ghent University, Belgium
[5]Serbian Academy of Sciences and Arts, Belgrade, Serbia

[*]e-mail: radomirovicmirjana@chem.bg.ac.rs

Tropomyosin has been recognized as one of the most common allergens among shellfish allergens. Sensitive and specific quantification of traces of allergens present in food samples is of critical importance for people with food allergies. This study thus aimed to develop a highly sensitive immuno-PCR method for detecting crustacean tropomyosin in foods. Method couples conventional sandwich ELISA assay with real-time PCR amplification of marker DNA. Monoclonal mouse anti-tropomyosin antibody was used as a capture antibody, while polyclonal rabbit anti-tropomyosin antibody served as a detection antibody in sandwich ELISA. A double-stranded amino-DNA molecule of 77 base pairs was covalently conjugated to a secondary goat anti-rabbit antibody and subsequently amplified and quantified by real-time PCR. Tropomyosin was quantified using highly purified natural shrimp tropomyosin as standard. The sensitivity of immuno-PCR for quantification of tropomyosin was increased by up to 20-fold compared to ELISA, demonstrating accuracy as low as 19.8 pg/mL. Recovery of tropomyosin in vegetable soup as a food matrix was in the 87.7–115.6% range, with relative standard deviations in the 5–24.5% range. Tropomyosin was also quantified in the commercially available food products. Developed immuno-PCR technique thus shows the potential to be a method of choice for specific and ultrasensitive detection of tropomyosin in food samples, with the final aim of reducing risks of accidental food contamination.

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