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Optimization of extraction conditions of tropomyosin from Mediterranean mussel and its quantification by developed ELISA

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Tropomyosin (TPM) is a major allergen among different shellfish species. Developing sensitive, specific, and reliable methods for quantifying TPM in food products is crucial for persons allergic to shellfish. Commonly used extraction buffers often show shortcomings in their extraction efficiency, which is why sometimes the presence of some allergens can be overlooked in the biological material. Therefore, this work aimed to optimize Mediterranean mussel TPM extraction conditions and develop a sandwich ELISA method for TPM quantification. Several extraction buffers were tested for their efficiency in recovering proteins from fresh frozen and cooked mussels during 2 and 24 hours of extraction. The protein content was quantified using the Bradford protein assay. Protein components of soluble extracts were profiled using SDS-PAGE. TPM presence in soluble extracts was confirmed by Western blot using both monoclonal and polyclonal anti-TPM antibodies. Sandwich ELISA was developed and used to quantify TPM content. None of the extraction buffers showed a significant difference in total protein content between 2 and 24 hours of extraction, indicating that 2 hours is sufficient for protein recovery in both raw and cooked mussels. Significantly fewer proteins were extracted from cooked mussels compared to raw mussels. Densitometrically estimated TPM concentrations indicate that PBS containing 1M NaCl (PBSN) extracts around 40% more TPM than PBS. Carbonate buffers extract even three times higher amounts of TPM than traditionally used extraction buffer PBS. Developed sandwich ELISA has shown not to be reliable for quantifying TPM from mussels, significantly underestimating its concentration, as concluded by comparing TPM concentrations obtained by ELISA with those obtained densitometrically. Therefore, Western blot has been used as an alternative method for mussel TPM quantification. The linear range for TPM quantification using Western blot was between 1.25 and 10 µg/ml. TPM concentrations in mussel extracts estimated using Western blot correlated well with those calculated by densitometric gel analysis. Further work will be aimed at improving the sensitivity of the presented methods and developing new methods for TPM quantification.

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