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Comparative digestomics of Tropomyosin of vertebrates and invertebrates in real food matrix

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Shellfish, is a highly nutritive food resource in the world, but also among the eight allergic food groups accounting for approximately 90% of all immunoglobulin E food allergies worldwide [1]. This work focuses on the only well-recognized major allergen muscle protein tropomyosin(TM) that is responsible for cross reactivity between shellfish and other invertebrates [2]. By contrary, TM of vertebrates (chicken, pig, cow) is not a prominent allergen. The stability of food allergens to digestion is an important factor contributing to their allergenicity. Most in vitro digestibility studies are based on the protein extract rather than whole food matrix thus overlooking its effect on TM stability [3]. Our objective was to primarily test the pepsin digestibility of invertebrates and vertebrates (raw and thermally treated based on their real life consumption modes) mimicking the gastric digestion under standardized conditions. To closely observe and compare the vertebrates' and invertebrates' TM stability, we aimed to perform the specific antibody based western blot analysis with two primary antibodies; ❶Rabbit anti shrimp TM antibody (invertebrates), and ❷ Rabbit anti human TM antibody (species reactivity to vertebrates).

Methods: Thermal treatment of selected samples to compare TM heat stability, Standardized static in vitro methods of simulated gastric digestion[4] for the evaluation and comparison of TM resistance to pepsin, Sodium Dodecyl Sulfate-Polyacryl amide Gel Electrophoresis (SDS-PAGE) of digesta supernatant under reducing and non-reducing conditions to quantify proteins and compare thermally treated invertebrates and vertebrates protein profiles focusing on TM, specific antibody based semi dry Western blot analysis.

Results and discussions: SDS-PAGE analysis of vertebrates and invertebrates' samples showed a range of proteins in varied amounts between 10-250 kDa. Depending upon samples, varied numbers of prominent protein bands were observed including the distinct bands corresponding with the molecular weights of TM(37-39kDa). In agreement with publications, TM was, indeed, resistant against pepsin digestion as well as thermal treatment prominently in case of invertebrates. This was confirmed upon Ab based Western blot analysis. Our results show that, upon thermal treatment, TM is partially degraded as is observed in case of raw and cooked beef electrophoretic profile as well as WB analysis. Significantly, upon pepsin digestion, TM (allergen) is completely degraded in vertebrates in contrast to the invertebrates' TM (which is pepsin resistant and heat stable).

This result provides an insight on the differences in digestibility of allergenic versus non-allergenic TM in real food matrix and upon thermal treatments of solid food samples.

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