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Matrix effect on food allergen detection – A case study of fish parvalbumin

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ABSTRACT

Two fish parvalbumin models were established to study relationships among matrix effect, extractability, and thermostability during *in vitro* immunodetection using two parvalbumin-specific monoclonal antibodies (3E1 and PARV19). Our results illustrated that matrix-induced thermal instability of parvalbumin was due mainly to physical (hydrophobic effect) and chemical (thiol-disulfide interchange) interactions. The addition of sodium dodecyl sulfate (SDS, surfactant), β-mercaptoethanol (reducing agent) or ethylenediaminetetraacetic acid (EDTA, metal chelator) during sample preparation could not only increase the extractability of parvalbumin but also enhanced its immunodetection. Our findings demonstrated excess EDTA completely chelated Ca^{2+} in parvalbumin and rendered it undetectable using PARV19 (a Ca^{2+}-dependent antibody). Overall, our resulted showed that matrix effect on *in vitro* analyte quantification cannot be underestimated. Any false negative or positive results could lead to severe or life-threatening allergic reactions.

1. Introduction

To prevent the occurrence of undeclared food allergenic residues, monoclonal antibody (mAb) based immunoassays have been developed for the *in vitro* detection of allergenic protein residues in processed foods (Jayasena et al., 2015). One of the critical factors in evaluating the quality of an immunoassay is whether the target analyte detected in sample extracts correctly reflect its content in tested products. It should be noted that matrix effects on the *in vitro* target analyte quantification cannot be underestimated and should not be ignored. According to the International Union of Pure and Applied Chemistry (IUPAC) (Guilbault & Hjelm, 1991), matrix effect is defined as "the combined effect of all components of the sample other than the analyte on the measurement of the quantity."

Currently, only a few studies have discussed matrix effects on the extractability of target analytes during *in vitro* immunodetection (Downs & Taylor, 2010; Gomaa, Ribereau, & Boye, 2012; Montserrat et al., 2015). The weak extractability of target analytes can lead to false negatives, which may result in the incorrect conclusion. For example, there has been a controversy about the immunoreactivity of fish parvalbumin using a commercial anti-parvalbumin mAb, PARV19 (Sigma-Aldrich Co., St. Louis, MO, USA). As the major fish allergen (Van Do, Elsayed, Florvaag, Hordvik, & Endresen, 2005), parvalbumin reacts with immunoglobulin (Ig) E from more than 90% of fish allergic individuals (Bugajska-Schretter et al., 1998). Parvalbumin is a water-soluble acidic intracellular Ca^{2+} binding protein, with a molecular weight (MW) of 10–13 kDa (Lopata & Lehrer, 2009). It is well known that parvalbumin content is different among various fish species (Kuehn, Swobode, Arumugam, Hilger, & Hentges, 2014). Even within the same species, parvalbumin is not evenly distributed in fish muscles (Lee, Nordlee, Koppelman, Baumert, & Taylor, 2012). It was reported that parvalbumin was immunodetected by Western blot (WB) using PARV19 in heated (95 °C for 15 min) Atlantic salmon, cod, orange roughy, and rainbow trout while it could not be detected in heated gummy shark (Saptarshi, Sharp, Kamath, & Lopata, 2014). However, another WB study showed that using the same sample preparation and immunodetection method, parvalbumin could not be detected in heated Atlantic salmon, cod, mahi-mahi, and orange roughy, but was immunodetectable in heated gummy shark and whiting (Sharp et al., 2015). When the heating conditions changed to 100 °C for 20 min, parvalbumin was not immunodetectable in cod, rainbow trout, and whiting; however, it was detected in mahi-mahi and orange roughy using PARV19-based indirect enzyme-linked immunosorbent assay (ELISA) (Gajewski & Hsieh, 2009). In order to explain this inconsistent phenomenon, two parvalbumin models were used in this study to demonstrate that matrix effect significantly impacts *in vitro* detection of food allergens. This study aims to investigate the relationship among matrix effect, extractability, and thermostability of fish parvalbumin during *in vitro* immunodetection. In this study, thermostability is defined as the capability of a target protein to retain its solubility,

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molecular integrity, and immunoreactivity after heat treatment.

2. Materials and methods

2.1. Materials

Fresh Atlantic salmon (*Salmo salar*) fillets were purchased from a local store (Tallahassee, FL, USA). Certified whole flathead grey mullet (*Mugil cephalus*) fishes were kindly provided by the Fish and Wildlife Research Institute (Eastpoint, FL, USA) at the Florida Fish and Wildlife Conservation Commission (FWC). Upon receiving, whole mullet fish was deboned. Boneless fish meats from both species were cut into small pieces, vacuum packed and stored at −80 °C until use. One anti-parvalbumin mAb, 3E1, was previously developed in our laboratory (Gajewski & Hsieh, 2009). Two commercial antibodies, PARV19 (P3088) and goat anti-mouse IgG (Fc specific) horseradish peroxidase (HRP) conjugate (anti-IgG-HRP, A2554), were purchased from Sigma-Aldrich. All chemicals and reagents were of analytical grade. All solutions were prepared using deionized (DI) water from a NANOpure DIamond ultrapure water system (Barnstead International, Dubuque, IA, USA).

2.2. Model sample preparation

Two sample models were established to study the matrix effect on thermostability of parvalbumins from mullet and salmon (Fig. 1). *Model 1* was the crude fish protein extracts (PE). *Model 2* was the purified parvalbumin (PP). Unless otherwise specified, all subsequent operations were performed at 4 °C. All samples were homogenized at 11,000 rpm for 2 min at room temperature (RT) using an ULTRA-TURRAX T25 basic homogenizer (IKA Works, Inc., Wilmington, NC, USA) and were centrifuged at 20,000*g* for 15 min. All heated samples were prepared at 100 °C/600 rpm using a thermomixer (Eppendorf, Hamburg, Germany). Each heated sample was weighed to confirm whether its mass was lost during heat treatment. If so, it was reprepared. All samples were prepared and analyzed on the same day.

For *Model 1*, the PE samples were prepared according to Gajewski and Hsieh (2009) with modifications (Fig. 1). Half-thawed minced mullet and salmon fillets were thoroughly mashed by hand and mortar. 2 g of each meat was then mixed with 4 mL of ice-cold DI water. After homogenization and 2-h end-over-end rotation, the homogenate was centrifuged. The supernatant (i.e., unheated PE) was filtered through a filter paper (Whatman, Piscataway, NJ, USA) and a 0.8-μm syringe filter (Thermo Fisher Scientific Inc., Fair Lawn, NJ, USA). For heated

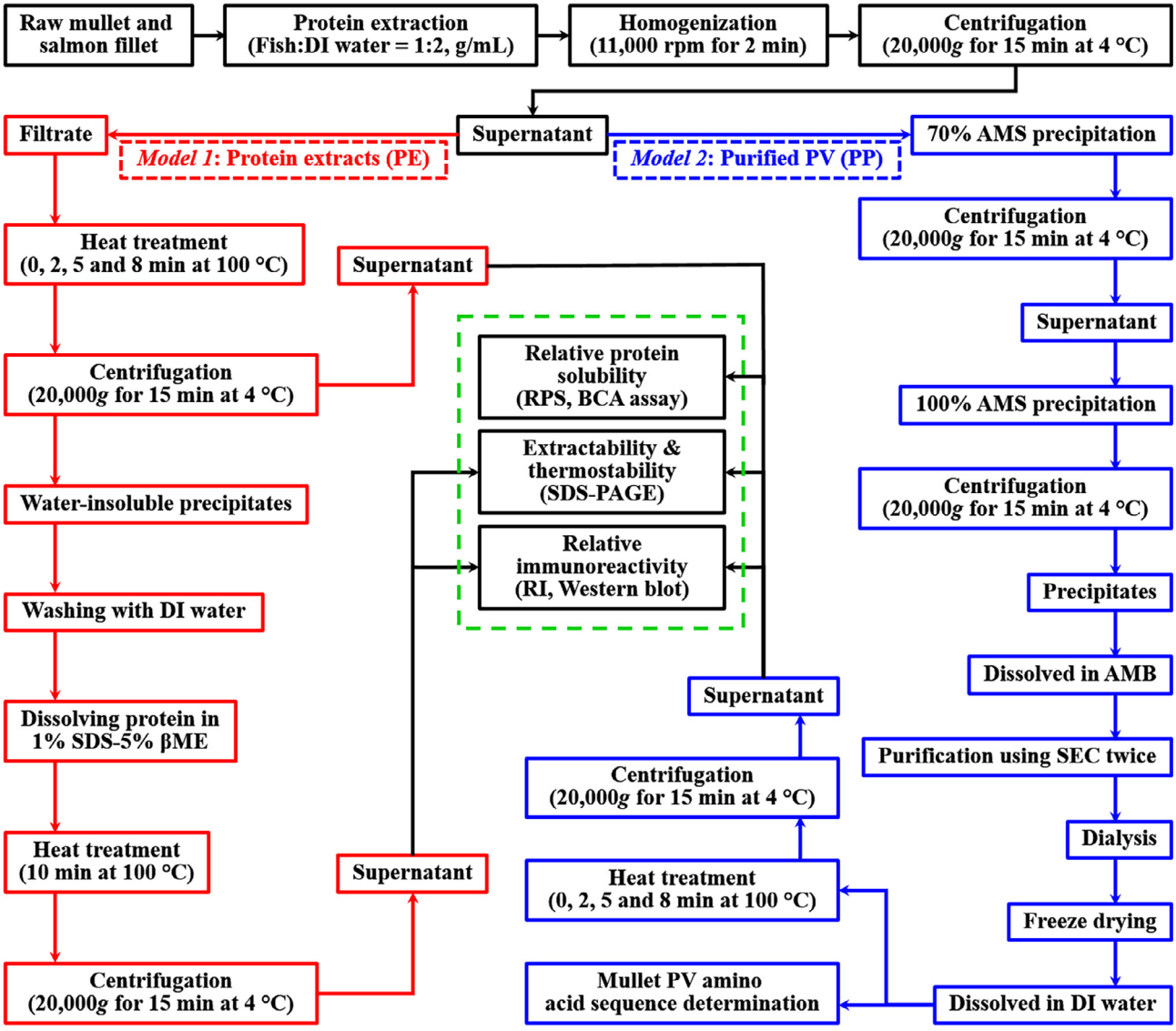

Fig. 1. Schema of sample preparation using DI water in two models.