

Estimating the quantity of egg white and whey protein concentrate in prepared crabstick using ELISA

Zachary H. Reed, Jae W. Park *

Oregon State University, Seafood Laboratory, 2001 Marine Drive Rm 253, Astoria, OR 97103, United States

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ABSTRACT

Indirect enzyme-linked immunosorbent assays (ELISA) technique was used to identify and quantify the use of dried egg white (DEW) and whey protein concentrate (WPC) in crabsticks. The use of SDS–PAGE for the quantification of protein additives has had limited success due to the high shear and high temperature processes of surimi crabstick. Monoclonal (anti-heat-denatured ovalbumin) and polyclonal (anti- β -lactoglobulin) antibodies were used. Antibodies showed no significant cross-reactivity with non-target crabstick proteins. An optimised extraction solution of 10% SDS and 2.5% 2-ME yielded high extractability with improved consistency. Quantification of DEW and WPC was achieved using the optimised extraction solution and indirect ELISA. Estimated DEW values were within 7% of actual values, WPC samples were within 17%. Inter-assay coefficients of variance for DEW ranged from 0.9% to 3.1% and those of the WPC were 1.0–8.0%.

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1. Introduction

The crabstick industry does not have a standard of identity established for protein additives such as dried egg white (DEW) and whey protein concentrate (WPC). A lack of standard of identities can lead to increased use and/or abuse of protein additives in the crabstick formulation, which decreases surimi quantity and correlates to lower quality product if the usage is not properly optimised. Surimi refers to refined fish myofibrillar proteins used as an intermediate product in many seafood flavoured products. Surimi is produced through heading, gutting, mincing, washing, dewatering and concentrating the fish myofibrillar proteins (Lee, 1984; Park & Lin, 2005). The concentrated myofibrillar protein is mixed with cryoprotectants such as sugar, sorbitol, and polyphosphates which serve to stabilize the fish myofibrillar protein during frozen storage (Park & Lanier, 1987; Park, Lanier, & Green, 1988). Surimi is comminuted with other ingredients such as starch, DEW, WPC, and other flavours in the production of crab-flavoured seafood (crabsticks). DEW has been used extensively as a functional food ingredient because of its gelling and foaming properties (Mine, 1995). β -Lactoglobulin (β -LG) is largely responsible for the physicochemical properties as well as the functional behaviour of food products that contain whey protein (Foegeding, Davis, Doucet, & McGuffey, 2002). Burgarella, Lanier, Hamann, and Wu (1985) showed that DEW and WPC can be used in low concentrations to provide an additive effect of increased gel strength and deformability.

One method used to quantify protein additives in crabstick is SDS–PAGE coupled with gel densitometry (Reed & Park, 2008). We found that SDS–PAGE was useful for detection of DEW but was not appropriate for the quantification of DEW. Enzyme-linked immunosorbent assays (ELISA) take advantage of the interaction between proteins and antibodies and can be used to qualify and quantify protein additives in food. Commercial ELISA kits are available for the detection of egg, milk, wheat, buckwheat, and peanut proteins. It is mandatory for these five allergens to be labelled in Japan (Matsuda et al., 2006) and in most countries including United States. Due to the severity of some food allergies the use of ELISA has become widespread for detection of allergenic proteins (Faeste, Egaas, Lindvik, & Lovberg, 2007; Fuller, Goodwin, & Morris, 2006). ELISA has also been shown to be effective in quantifying egg white in processed pork products (Leduc et al., 1999). Dupont et al. (2006) have developed an ELISA for bovine lactoferrin to determine lactoferrin concentrations in milk, whey, and cheese. Ovalbumin (OA) is the most abundant of egg white proteins, comprising 54% of the total proteins (Stevens, 1991). Of the whey proteins in bovine milk, β -lactoglobulin (β -LG) is the most abundant (Farrell et al., 2004).

Extraction of protein from the cooked gel sample is one of the most critical steps in obtaining a clean and reproducible ELISA assay. Commonly, Tris-buffered saline (TBS) and phosphate buffered saline (PBS) have been used to extract protein from the sample matrix. Asensio et al. (2003) used 0.85% saline solution to extract proteins from grouper, wreck fish and Nile perch filets. Hefle, Jeanniton, and Taylor (2001) used PBS to extract proteins from pasta for the detection of egg residues in food, while Holden, Fæste,

* Corresponding author. Tel.: +1 503 325 4531; fax: +1 503 325 2753.

E-mail address: jae.park@orst.edu (J.W. Park).

and Egaas (2005) used 0.1 M Tris, 0.5 M glycine at pH 8.7 for the extraction of lupine from several baked goods. Preliminary studies revealed that, when crabstick samples were extracted with PBS alone either overnight at room temperature or at 90 °C for an hour, results were not reproducible. Due to the strong gel matrix of high protein content, different extraction methods were investigated.

Watanabe et al. (2005) investigated the use of an extraction solution containing sodium dodecyl sulphate (SDS) and 2-mercaptoethanol (2-ME) for the detection of raw and processed egg. In their study they found the total protein extracted using TBS could be increased 10–100-fold with the addition of 1% SDS and 7% 2-ME to the TBS extraction solution. Ochiai and Watabe (2003) found that using an extraction solution of urea, SDS, and 2-ME, allowed for species identification of processed fish products.

The overall goal of this study was to develop an indirect ELISA assay for the qualification and quantification of dried egg white and whey protein concentrate when used as an additive to a cooked and pasteurised crabstick product. In addition, we attempted to develop an optimised extraction method for fish protein gels with strong texture.

2. Materials and methods

2.1. Food products and chemicals

Alaska pollock (*Theragra chalcogramma*) surimi (FA grade, 10 kg blocks), were obtained from Western Alaska Fisheries (Seattle, Wash., USA) and stored frozen at –18 °C. The 10 kg blocks were cut into approximately twenty 1 kg blocks, individually vacuum packed, and stored at –18 °C until used for surimi crabstick paste preparation. Ingredients used for surimi crabstick paste preparation were NaCl (Morton Iodised Salt, Morton International, Inc., Chicago, Ill., USA), corn starch (Corn Products International, Westchester, Ill., USA), wheat starch (Midsol 50, MGP Ingredients, Inc., Atchison, Kans., USA), modified waxy maize starch (Polartex 06727, Cargill, Inc., Cedar Rapids, Iowa, USA), sugar (used to represent all flavour components) (Pure Cane Sugar, C&H Sugar Company, Inc., Crockett, Calif., USA), and culinary tap water. The protein additives were dried egg white (DEW) (K-200, Henningsen Foods, Omaha, Nebr., USA) and whey protein concentrate (WPC 8600, Hilmar Ingredients, Hilmar, Calif., USA). All other chemicals were reagent grade.

2.2. Antibodies

Affinity purified rabbit polyclonal antibodies produced against bovine β -lactoglobulin (pAb anti- β -LG) and goat anti-mouse IgG coupled with horse radish peroxidase (HRP-anti-mIgG) were obtained from Bethyl Laboratories (Montgomery, Tex., USA). Mouse monoclonal antibodies against heat denatured hen ovalbumin (Ab anti-H-OA) were purchased from Abcam, Inc. (Cambridge, Mass., USA). Goat anti-rabbit IgG coupled with HRP (HRP-anti-rIgG) was purchased from Sigma-Aldrich, Inc. (St. Louis, Mo., USA). All antibodies were delivered at a concentration of 1 mg/mL. Antibodies were tested against a serial dilution of purified ovalbumin (OA) or β -lactoglobulin (β -LG) which produced a linear response. The antibodies were also tested against crabsticks. When tested against crabsticks, antibodies showed no reactivity with proteins other than the target protein.

2.3. Sample preparation

2.3.1. Basic surimi paste batches

Crabsticks were made with base ingredients including: surimi (40%), water (45%), salt (2%), starches (8%), and sugar (5%). Three

formulations for crabstick were made containing base ingredients and protein additives such as DEW only, WPC only, and 50/50 mixture of DEW/WPC. For each of the three formulations a total of nine batches per formulation were made. Six standard batches containing various protein additives were made (0.00%, 0.25%, 0.50%, 1.00%, 1.50%, and 2.00% protein additives). Three additional verification batches were also made (0.375%, 0.750%, and 1.750% protein additives). For batches with protein additives, starch was replaced in a 1:1 ratio (w/w) by protein additive resulting in an equal moisture concentration of approximately 75%. Final batch sizes were approximately 1300 g. Frozen surimi was allowed to thaw at room temperature (≈ 23 °C) for approximately 1 h and then cut into small pieces. All chopping was performed using a Stephan vertical vacuum cutter (model UM 5 universal, Stephan Machinery Co., Columbus, Ohio, USA). Surimi pieces were added to the chopping bowl and chopped at 1800 rpm for 1 min. Salt (26 g) was then added to the chopped surimi and chopping continued at 1800 rpm for 1 min. Following the integration of the salt, 65 g of sugar was added in addition to starch (50:40:10 corn:wheat:modified waxy maize), protein additive when required, and water followed by chopping at 1800 rpm for 1 min. A vacuum of 40–60 kPa was applied to the surimi paste and chopping continued for 3 min at 3000 rpm. Surimi paste was then placed in a plastic bag and refrigerated until samples were cooked.

Approximately 20–25 g of labelled surimi paste was placed into a sheet mould (stainless steel 25 cm $\times$ 7.5 cm $\times$ 1.4 mm) formed on top of a piece of aluminium foil, which had been sprayed with no-stick cooking spray (Pam® Original, ConAgra Foods, Inc., Omaha, Nebr., USA). Excess raw surimi paste was vacuum packed and frozen at –80 °C. Food grade plastic film was laid over the top of the paste and a stainless steel tube roller (11.5 cm $\times$ 4 cm dia.) was used to evenly spread out the paste to form a crabstick sheet. After the film was removed the aluminium foil containing the crabstick sheet was cooked in a steam bath (93 °C) for 90 s. After initial cooking, crabstick sheets were cut in half, wrapped in plastic film and then placed back in the 93 °C steam bath for 30 min to simulate commercial pasteurisation. Pasteurised samples were submerged in ice water to cool for 15 min. If samples were not to be used within 48 h they were frozen at –80 °C until needed.

For the verification process, three batches per formulation were made (0.375%, 0.75%, and 1.75% protein additive) and the protein concentrations were estimated using the linear equation produced from the five standard batches (0.25%, 0.50%, 1.00%, 1.50%, and 2.00% protein additives). Using Microsoft Excel software the standard curves were obtained by plotting the absorbance at 450 nm vs the protein additive concentrations of 0.25%, 0.50%, 1.00%, 1.50%, and 2.00%. The standard curve was fitted to a linear equation ($y = mx + b$), where y = absorbance at 450 nm, m = slope of the line, x = protein additive %, and b = intercept of the line. Calculation of the verification samples were given by the following equation: $x = (y - b)/m$.

2.4. Optimised sample extraction

During our preliminary experiments, we realised the optimisation of the extraction solution was extremely important to obtain consistent data. In a preliminary optimisation step 25 extraction solutions were tested. Extraction solutions contained various levels of SDS (2.5%, 5%, and 7.5% w/v), Tween 20 (0.05%, 0.075%, and 0.1% v/v) and 2-ME (5%, 7.5%, and 10% v/v). All extraction solutions were incubated for 1, 2, or 3 h at 90 °C (results not shown). A final optimised extraction protocol was achieved using 10% SDS w/v and 2.5% 2-ME v/v. Tween 20 was determined to have no significant effect on the extraction of the protein from the crabstick samples. This may be due to the much more substantial amount of SDS used as both are detergents. Samples used for the standard curve pro-

duced a linear response which then allowed for the estimation of the DEW and WPC content of the verification batches.

Approximately 2 g of sample was accurately weighed into a 50 mL centrifuge tube and 20 mL of solution containing 10% SDS and 2.5% 2-ME was added. Samples were homogenised for 1 min at speed 4 (Powergen 700, Fisher Scientific, Pittsburgh, Pa., USA) followed by extraction for 1 h at 90 °C. Extracted samples were centrifuged at 7796g (Sorvall RC-5B, DuPont Co., Newton, Conn., USA) for 20 min. The supernatant was filtered (70 mm dia. # 541, Whatman International Ltd., Maidstone, UK) into a 25 mL volumetric flask and brought to volume using additional 10% SDS and 2.5% 2-ME solution.

2.5. Indirect ELISA

Ovalbumin (OA) from chicken egg white, grade VI (Sigma–Aldrich, Inc., St. Louis, Mo., USA) and β -lactoglobulin (β -LG) from bovine milk (Sigma–Aldrich, Inc., St. Louis, Mo., USA) were serially diluted using PBS, containing 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer (Omnipur 10 $\times$ PBS Concentrate, EMD Chemicals, Inc., Gibbstown, NJ, USA) and assayed in both the OA and β -LG ELISA checking for linearity and cross-reactivity. OA and β -LG were solubilised in 5% SDS (w/v) and heated at 90 °C for 1 h. Total protein content was determined using the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). Samples were serially diluted with PBS to 25 ng/mL and tested in triplicate. Indirect ELISA results were given as the average of the three serial dilutions.

Crabstick samples were serially diluted in PBS to a final dilution of 1:10,000. Polystyrene 96 well microtiter plates (Nalge Nunc International, Rochester, NY, USA) were coated with 100 μ L of serially diluted samples (6 wells/sample) and were incubated overnight at 4 °C. The plates were washed three times with PBS containing 0.5% (v/v) Tween 20 (PBS-T) and blocked with 100 μ L of Superblock[®] blocking buffer in PBS with 0.05% Tween 20 (Thermo Scientific, Rockford, Ill., USA) at 37 °C for 30 min. After the plates were washed three times with PBS-T, 100 μ L of diluted (1:5000, 1 mg/mL) Ab anti-H-OA or pAb anti- β -LG antibodies were added to the wells and incubated at 37 °C for 1 h. After three PBS-T wash cycles, 100 μ L of diluted (1:5000, 1 mg/mL) HRP-anti-mIgG when detecting Ab anti-H-OA and HRP-anti-rIgG when detecting pAb anti- β -LG were added to each well and incubated at 37 °C for 30 min. Plates were washed three times using PBS-T after which 100 μ L of 3,3',5,5'-tetramethylbenzidine, 1-Step Ultra TMB ELISA (Thermo Scientific, Rockford, Ill., USA) was added and allowed to develop for 8 or 12 min at room temperature, for the DEW and WPC samples, respectively. Colour development was stopped by the addition of 100 μ L of 2 N sulphuric acid per well. The plate was read at 450 nm using a Biolog Microlog (Biolog, Hayward, Calif., USA).

2.6. Immunoblotting

SDS–PAGE was performed as outlined by Laemmli (1970) using a 12.5% (% T) separating gel and a 4% (% T) stacking gel. The crabstick samples used for SDS–PAGE and immunoblotting contained both DEW and WPC. A 0.5 mL aliquot of extracted sample, that was used for the indirect ELISA, was mixed with 0.5 mL of Laemmli sample buffer (0.6 mL 1 M Tris–HCl (pH 6.8), 5.0 mL glycerol, 2.0 mL 10% (w/v) SDS, 0.5 mL 2-ME, 1.0 mL 1% (w/v) bromophenol blue, 0.9 mL H₂O). Sample and buffer were vortexed and then heated for 3 min at 90 °C to disrupt any disulphide bonds. Samples were separated using a Mini-Protean III Cell (Bio-Rad, Hercules, Calif., USA) according to the operating manual.

After electrophoresis the gels were removed and equilibrated in Towbin solution (25 mM Tris, 192 mM glycine, 20% methanol (v/v) at pH 8.3) for 20 min and transferred to a nitrocellulose membrane

(Bio-Rad, Hercules, Calif., USA) using a Mini Trans-Blot[®] Electrophoretic Transfer Cell (Bio-Rad, Hercules, Calif., USA) using a slight modification of an earlier published method (Towbin, Staehelin, & Gordon, 1979). The membrane was blocked using Superblock[®] blocking buffer in PBS with 0.05% Tween 20 (Thermo Scientific, Rockford, Ill., USA) for 30 min at 25 °C. The blocked membrane was washed three times for 5 min using PBS-T. The membrane was incubated with either Ab anti-H-OA or pAb anti- β -LG (1:15,000) for 60 min at 25 °C after which the membrane was washed three times. The membrane was incubated with HRP-anti-mIgG when detecting Ab anti-H-OA and was incubated with HRP-anti-rIgG when detecting pAb anti- β -LG antibodies (1:15,000) at 25 °C for 60 min followed by three additional wash cycles. Bands were visualised using 1-Step[™] TMB-Blotting (Thermo Scientific, Rockford, Ill., USA). Development was stopped by the addition of dd water. Molecular weight was determined by comparison to the Kaleidoscope[™] protein standard (Bio-Rad, Hercules, Calif., USA) also run in each SDS–PAGE.

2.7. Statistical methods

For determination of the inter-assay precision, the mean coefficients of variation (CV) were based on three extractions performed on separate days. Each day an extraction was performed and the extract was assayed in triplicate on the same day. All calculations were done using Microsoft Excel (Microsoft Corporation, Redmond, Wash., USA). Each assay contained six replicates of each sample. The lower limit of detection (LLD) for the indirect assay was given as three times the standard deviation plus the background of blank wells. The lower limit of quantification (LLQ) was taken to be the lowest point (0.25% protein additive) of the standard curve.

3. Results and discussion

3.1. Optimised sample extraction

Extracting the proteins with 10% SDS and 2.5% 2-ME for 1 h at 90 °C was found to be the optimum condition yielding reproducible and reliable data for the ELISA assay. Fig. 1 shows the increased accuracy and decreased variation obtained when the optimised extraction solution was used. The calculated protein was determined using the standard curve created from the standard crabstick batches. In Fig. 1A, it is shown that the calculated % protein for DEW (calculated protein divided by protein added) ranges from 91.5% to 120.1% for the pre-optimised extraction solution (1% SDS (w/v) and 5% 2-ME (w/v)). It is our opinion that the addition of SDS and 2-ME was successful in disrupting the bonds formed during gelation. The gel matrix formed during the cooking of the crabsticks is believed to have caused steric hindrance during adsorption onto the ELISA plate. Due to the steric hindrance, some of the antibody epitopes were not available for interaction with the antibody. For the optimised extraction solution the range narrows to 100.0–101.7%. The standard deviation for the pre-optimised solution ranged from 4.6% to 24.0% and that of the optimised solution was 4.0–6.3%. The WPC extraction optimisation showed a similar trend. The pre-optimised calculated % protein ranged from 85.9% to 134.4% with a standard deviation range of 5.7–40.0%. The calculated % protein for WPC using the optimised extraction solution ranged from 95.6% to 116.7% with a standard deviation ranging from 1.7% to 11.4%.

3.2. Reactivity testing and immunoblotting

Purified OA and β -LG proteins were examined for reactivity and cross-reactivity with the Ab anti-H-OA and pAb anti- β -LG. In

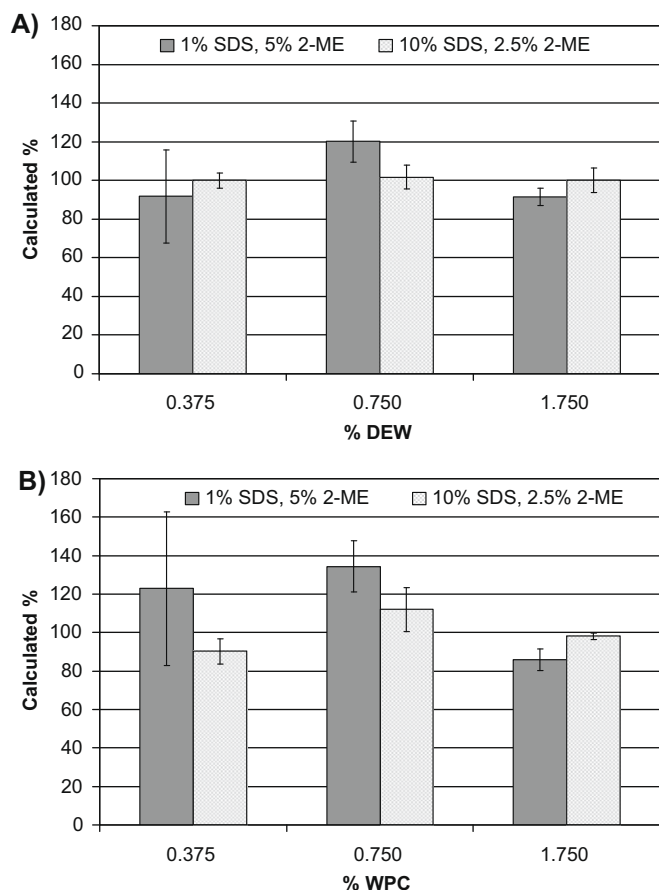


Fig. 1. Protein extraction using pre-optimised solution (1% SDS and 5% 2-ME) and optimised solution (10% SDS and 2.5% 2-ME) (A) crabstick containing DEW (B) crabstick containing WPC. The calculated protein was determined using the standard curve created from the standard crabstick batches.

Fig. 2A, there is a linear response for OA passively absorbed to the microtiter plate and incubated with Ab anti-H-OA, while virtually no binding was observed with β -LG when incubated with the same Ab anti-H-OA. In **Fig. 2B**, a linear response was observed for β -LG when incubated with pAb anti- β -LG. Again virtually no response was made for the OA incubated with pAb anti- β -LG. Immunoblotting of crabstick samples for DEW detection using Ab anti-H-OA showed similar patterns to those produced from purified ovalbumin and DEW only samples (**Fig. 3A**). **Fig. 3B** shows similar results for samples tested for the presence of β -LG using pAb anti- β -LG.

3.3. Lower limit of detection vs lower limit of quantification (LLD vs LLQ)

Blank wells (36 total) were tested using Ab anti-H-OA with an average absorbance of 0.044 ± 0.006 and a LLD of 0.062 absorbance. The LLQ for the ELISA assay was calculated as the lowest point on the standard curve using 0.25% protein additives (16.5 and 12.4 ng/mL for DEW and WPC, respectively). The average absorbance value for the 0.25% DEW sample was 0.361 ± 0.034 . The control sample (0% DEW) had an average absorbance of 0.101 ± 0.021 which was 3.6 times lower than that of the 0.25% DEW sample. The absorbance of the control sample was higher than the LLD but still below the LLQ. The average absorbance for the blanks were calculated using the standard curve the resulting values were negative showing that the blank values lie outside of the linear range of the standard curve. A similar trend was found using the blank values and standard curve for the WPC. The blank

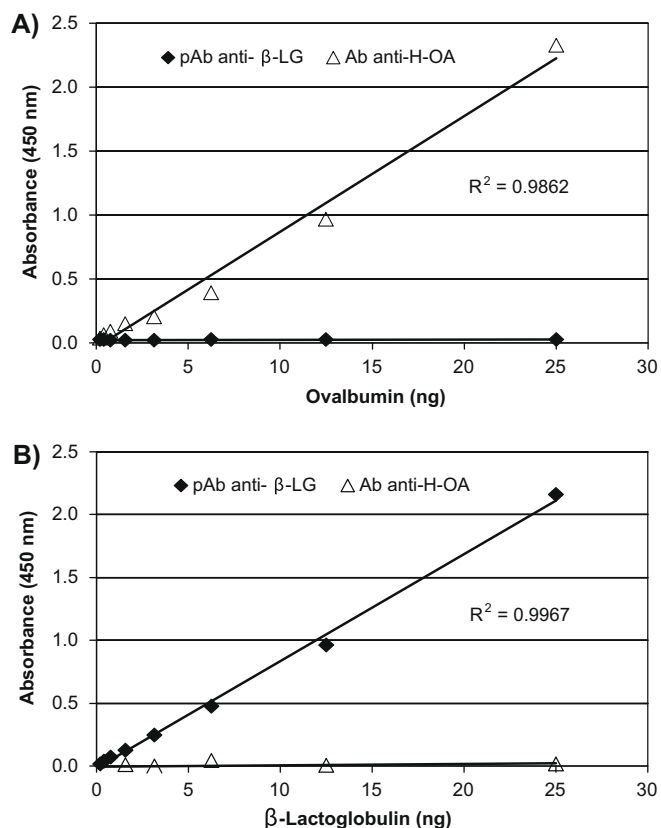


Fig. 2. Linear response of Ab anti-H-OA (A) and pAb anti- β -LG (B) tested against ovalbumin and β -lactoglobulin.

wells for WPC had an average absorbance of 0.057 ± 0.007 and a LLD of 0.078. The absorbance of the LLQ for WPC was 0.476 ± 0.037 . The control sample (0% WPC) had an average absorbance of 0.085 ± 0.008 . Again the control sample was slightly higher than the LLD but still much lower than the LLQ. The range for the standard curve was chosen so as to cover the most commonly added amounts of protein additive.

3.4. Indirect ELISA of DEW and WPC in crabstick

Using the most abundant proteins found in the respective protein additives helped in creating an accurate assay. Finding antibodies that will bind well with the heat-denatured form of OA and β -LG was a key for the success of the assay development. An indirect ELISA was used to qualify and quantify the use of DEW and WPC in prepared crabstick. Due to the highly processed nature of the crabstick, the proteins found in the sample have been completely denatured. Chemical (addition of salt) and physical (chopping and cooking) processing made it even more difficult to find the antibodies that could detect the denatured protein. The Ab anti-H-OA was produced to bind strongly with OA in denatured and modified forms such as heat-denatured and reduced. The antibody used to detect β -LG was created using native β -LG. **Table 1** shows the results of the verification batches for DEW, WPC, and their mixture (DEW/WPC). The calculated % protein was determined by dividing the determined protein % from the standard curve by the actual protein % added. Samples containing DEW had a calculated % protein that ranged from 98.8% to 106.6% and a standard deviation range of 2.5–6.3%. All calculated % protein values for the samples that contained DEW were within 7% of the actual added protein value. The WPC had a calculated % protein that ranged from 90.3% to 116.7% and a standard deviation range of

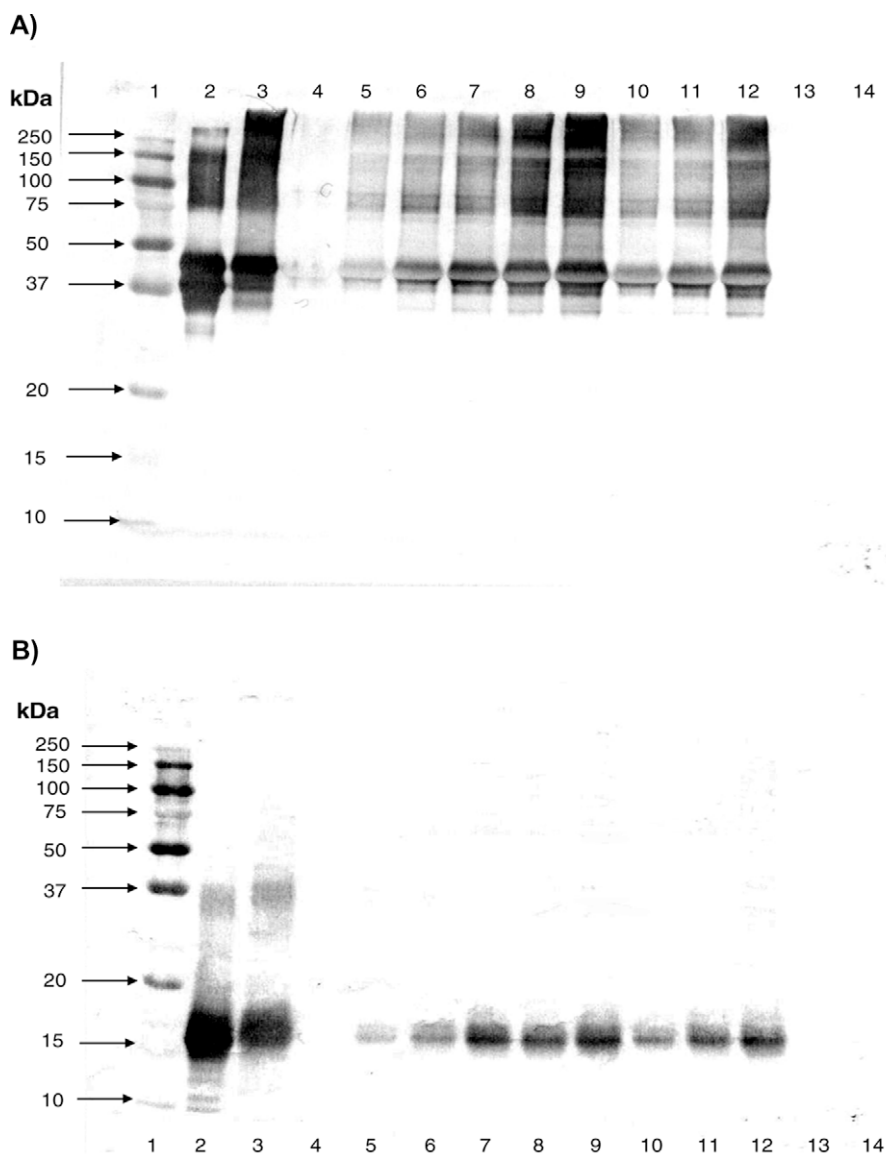


Fig. 3. Immunoblotting of DEW/WPC mixture tested using Ab anti-H-OA (A) and pAb anti-β-LG (B). (A) Lane (L) (1) Kaleidoscope marker, (2) ovalbumin, (3) DEW, lanes 4–12 crabstick batches (% DEW/WPC) (4) 0%, (5) 0.25%, (6) 0.5%, (7) 1.0%, (8) 1.5%, (9) 2.0%, (10) 0.375%, (11) 0.75%, (12) 1.75%, (13) β-LG, (14) WPC. (B) Lane (L) (1) Kaleidoscope marker, (2) β-LG, (3) WPC, lanes 4–12 crabstick batches (% DEW/WPC) (4) 0%, (5) 0.25%, (6) 0.5%, (7) 1.0%, (8) 1.5%, (9) 2.0%, (10) 0.375%, (11) 0.75%, (12) 1.75%, (13) ovalbumin, (14) DEW.

1.3–11.4%. All calculated % protein values for WPC samples were within 17%.

To understand the precision of the assay, the CV were analysed and reported in Table 2. Overall assay CV for DEW samples ranged from 0.9% to 3.1% while those for the WPC samples ranged from 1.0% to 8.0%. The difference in CV range for the two assays may be due, in part, to the nature of the antibodies used. The Ab anti-H-OA was developed against heat denatured OA. However the pAb anti-β-LG was created using the native form of β-LG. The difference in the CV may be because of increased avidity of the Ab anti-H-OA with denaturation. An antibody produced using heat denatured β-LG may possibly lead to an antibody with a stronger avidity for heat denatured β-LG.

Breton, Phan Thanh, and Paraf (1988) found that affinity purified antibodies raised against native ovalbumin had higher avidity against heat-denatured ovalbumin than against the native ovalbumin molecule. Rumbo, Chirido, Fossati, and Anon (1996) found that

avidity peaked and then began to decrease as heating time increased. This trend held true for samples tested between 70 and 100 °C. They attributed the decrease in avidity to an increase in OA unfolding. Luis, Perez, Sanchez, Lavilla, and Calvo (2007) showed that the use of native β-LG as an antigen, still allowed for the detection of highly denatured β-LG in food products including meat, bakery products, sauces, and snacks. Chicón, Belloque, Alonso, and López-Fandiño (2008) found that high pressure treatment of β-LG at 200 and 400 MPa actually increased the binding of β-LG-specific rabbit IgG antibodies when tested in an indirect ELISA format. According to Svenning, Brynhildsvold, Molland, Langsrud, and Vegarud (2000), β-LG antibody antigenicity was reduced due to the WPC processing. However, they stated that the reduction may be due to lower protein solubility. For WPC that was heated without any hydrolysis they showed 61% protein solubility and a 500-fold decrease in antigenicity. With pre-heated and hydrolysed WPC, they showed 100% protein solubility and a

Table 1
Protein additive estimation of verification batches using indirect ELISA.

	Added protein (%)	Determined protein (%)	Sample recovery	
			% ^a	CV (%) ^a
DEW	0.375	0.375	100.0	3.1
	0.750	0.763	101.7	2.9
	1.750	1.749	100.2	1.0
WPC	0.375	0.339	90.3	7.6
	0.750	0.840	112.0	5.3
	1.750	1.717	98.1	1.0
DEW/WPC ^b (mixture)	0.375	0.371	98.8	2.8
	0.750	0.799	106.6	0.9
	1.750	1.755	100.3	1.8
DEW/WPC ^c (mixture)	0.375	0.383	102.2	8.0
	0.750	0.875	116.7	3.4
	1.750	1.674	95.6	4.0

^a Sample recovery (%) and coefficient of variance (CV) as determined from three extractions done on three separate days in triplicate plates.

^b Sample were tested with Mab H-OA detection antibodies.

^c Sample were tested with Pab β -LG detection antibodies.

Table 2
Inter-assay coefficient of variance^a (%) by indirect ELISA.

	Added protein (%)	Inter-assay (% CV)
DEW	0.375	3.1
	0.750	2.9
	1.750	1.0
WPC	0.375	7.6
	0.750	5.3
	1.750	1.0
DEW/WPC ^b (mixture)	0.375	2.8
	0.750	0.9
	1.750	1.8
DEW/WPC ^c (mixture)	0.375	8.0
	0.750	3.4
	1.750	4.0

^a Inter-assay CVs are given as the mean of three extractions done on three separate days. Each extract from each extraction was assayed in triplicate on the same day.

^b Samples were tested with Mab H-OA detection antibodies.

^c Samples were tested with Pab β -LG detection antibodies.

10-fold decrease in antigenicity. Their study pointed out that β -LG antibody antigenicity was affected not only by processing conditions, but also by protein solubility.

4. Conclusions

Obtaining antibodies that would bind the target protein in the highly processed crabstick proved to be a key for the success of the assay development. Ab anti-H-OA and pAb anti- β -LG were shown to be highly specific for the target protein and showed no cross-reactivity with non-target proteins found in the prepared crabstick. An optimised solution for extraction was made with 10% SDS and 2.5% 2-ME. This optimised extraction solution demonstrated high reproducibility for the prepared crabsticks. An indirect ELISA assay was tested and proved as an effective method for the qualification and quantification DEW and WPC in a cooked and pasteurised crabstick. All three levels of DEW protein additive were estimated to less than 7% of the actual value and those of the WPC

were estimated to less than 17%. Accuracy of the WPC assay may be improved by producing an antibody from the heat denatured form of β -LG.

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