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ScienceDirect
Food Chemistry 107 (2008) 1–10
Food Chemistry
www.elsevier.com/locate/foodchem

Structure and functional properties of modified threadfin bream sarcoplasmic protein

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Received 23 December 2006; received in revised form 11 February 2007; accepted 30 March 2007

Abstract

Surimi wash-water contains up to 30% of protein from fish muscle that is currently underutilized. This paper describes the effect of acetylation, succinylation, trypsin hydrolysis or pre-heating at 55 °C on the emulsification and foaming properties of a threadfin bream sarcoplasmic protein (TBSP) model for wash-water protein. Multiple regression analysis showed that emulsification and foaming characteristics were differentially affected by TBSP surface hydrophobicity (S_0), solubility in water (S_W) and free amino group (fNH_2) concentration. Emulsification activity index (EAI) for TBSP was most enhanced by succinylation, whereas the foaming capacity (FC) was more effectively extended by trypsin hydrolysis. Structure–function relationships for emulsification were different from those associated with foaming or for ensuring the stability of these food dispersions. This study suggests that surimi wash-water protein functionality can be improved by protein modification. Further strategies may be needed to stabilize fish protein stabilized emulsions and foams.
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Keywords: Threadfin bream; Fish protein; Functionality; Chemical modification; Emulsification; Foaming

1. Introduction

Surimi wash-water recovers 20–35% of fish muscle protein, which is frequently discarded as waste. However, methods for the efficient recovery of fish process water proteins are becoming available, including, ultrafiltration (Jaoen & Quemeneur, 1992; Lin, Park, & Morrissey, 1995; Montero & Gomez-Guillen, 1998), flocculation by ferric chloride or chitosan–alginate (Marti, Roeckel, Aspe, & Kanda, 1994; Torres & Savant, 2001; Wibowo, Savant, Cherian, Savage, & Torres, 2005) and precipitation by ohmic heating (Benjakul, Morrissey, Seymour, & An, 1997, 1998). The technological properties of fish wash-water proteins are being investigated in order to facilitate their utilization as food ingredients. Protein extracts from Pacific whiting (Kim & Park, 2004; Lin et al., 1995), sardine (Kawai, Ohno, Inoue, & Shinano, 1995), arrow tooth flounder, herring and salmon (Sathivel et al., 2004, Sathivel, Bechtel, Babbitt, Prinyawiwatkul, & Patterson, 2005) have been described.

Threadfin bream (*Nemipterus hexodon*) is used for surimi manufacturing by the leading producer countries of south east Asia such as Japan, Thailand and India. Estimates show that Japan and Thailand each produce greater than 60,000 metric tonnes of surimi per year and 16,000–32,000 metric tonnes per year of surimi wash-water protein (Morrisey & Tan, 2000; Morrisey, Lin, & Ismond, 2005). Recent investigations have demonstrated that threadfin bream sarcoplasmic protein (TBSP) concentrate, selected

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0308-8146/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2007.03.075

as a model for threadfin bream surimi wash-water protein, possesses high nutritional value, with functional properties approaching those for milk proteins, such as β-lactoglobulin and caseinate (Krasaechol, Sanguandeekul, Duangmal, & Owusu-Apenten, 2005; Morioka, Nishimura, Obatake, & Shimizu, 1997). Further work is essential in order explore the full potential of TBSP as a functional ingredient.

Chemical, enzymatic or physical modifications are important approaches for optimizing protein functional properties. Attachment of well-defined chemical residues is also a valuable strategy for exploring protein structure–functionality relations (Ashi, Simpson, & Smith, 1996; Campbell, Raikos, & Euston, 2003; Davis & Williams, 1998; Djagny, Wang, & Xu, 2001; Hamada, 1994; Oliver, Melton, & Stanley, 2006; Panyam & Kilara, 1996; Roller & Dea, 1992). Succinylation, or partial hydrolysis by trypsin and chymotrypsin, improved the emulsification and foaming properties of whey proteins (Lakkis & Villota, 1992). Acylation and succinylation of canola protein enhanced water solubility, foaming capacity and emulsification. Such functionality changes were related to alterations in protein structure, including, protein dissociation, partial unfolding, altered isoelectric point, decreased enthalpy of denaturation and increased hydrophobicity (Gruener & Ismond, 1997a, 1997b). Succinylation of soybean protein isolate, following partial hydrolysis, improved emulsification and foaming characteristics (Achouri, Wang, & Xu, 1998). Fares, Landy, Guilard, and Voilley (1998) showed that succinylation alters protein flavour binding. Kosters et al. (2003) employed a variety of chemical modifications, with a view to enhancing food and medical applications of ovalbumin. A number of investigations have focussed solely on the effect of pre-treatment with proteases on food protein functionality (Kristinsson & Rasco, 2000; Panyam & Kilara, 1996). Partial enzymatic hydrolysis of soybean protein isolate, using papain, increased surface hydrophobicity, solubility and emulsification activity compared to the unmodified protein (Wu, Hattiarachy, & Qi, 1998). Treatment of amaranth protein with papain increased its solubility for use as a food ingredient (Scilingo, Ortiz, Martinez, & Anon, 2002). Partial hydrolysis of oat proteins by trypsin increased water solubility, water-holding capacity, emulsifying activity and foaming (Guan, Yao, Chen, & Zhang, 2007).

Based on evidence from the past literature, it was hypothesized that the functionality of TBSP could be altered by chemical, enzymatic or physical modification (Kristinsson & Rasco, 2000; Sikorski & Naczk, 1981). However, the magnitude or direction of any induced changes could not be predicted *ad-hoc* because specific protein groups respond differently to similar types of modification (Kinsella, 1976; Vojdany & Whitaker, 1994). In this study, TBSP was subjected to four well-defined types of chemical modification (acetylation, succinylation, trypsin hydrolysis and pre-heating) which are known to alter protein charge, hydrophobicity and water solubility. In addition, trypsin hydrolysis leads to reductions in protein molecular weight and viscosity. Five TBSP variants (including an unmodified control) were characterized in terms of water solubility (S_W), surface hydrophobicity (S_0), and the free amino group (fNH_2) concentration. The same samples were also subjected to functionality testing using standardized methods (Owusu-Apenten, 2004). Ultimately, it was hoped that this study would yield structure–functionality relationships from which it would be possible to select specific forms of protein modification to meet different application needs.

2. Materials and methods

2.1. Preparation of threadfin bream sarcoplasmic protein

Ornate threadfin bream (*Nemipterus hexodon)* was caught within the Indo-West Pacific region and frozen-on-board ship at −18 °C. The typical time to market was 30–45 days. Samples of fish were purchased from the fish market, Amphur Muang, Samut Sakhon Province, Thailand. Threadfin bream sarcoplasmic protein concentrate (TBSP) was prepared as described previously and freeze-dried for storage (Krasaechol et al., 2005; Morioka et al., 1997).

2.2. Chemical modification by acylation

Acylation of TBSP was performed as described for other proteins by Franzen and Kinsella (1976) with modifications. TBSP (2.0 g) was dispersed in distilled water (200 ml). Thereafter acetic anhydride (2.0 ml) or succinic anhydride (2.0 g) was added as 10 equal increments, with stirring. After each incremental addition, the pH of the mixture was adjusted to 7 or 8 with small additions of 3.5 M NaOH. Following acylation, the reaction mixture was stirred for 1 h and then dialyzed for 36 h against 0.01 M sodium phosphate buffer (pH 7.0) at 4 °C to remove excess acylation reagent. Modified TBSP was freeze-dried for storage. The extent of acylation was determined from the analysis of free amino acid residues (fNH_2), using trinitrobenzenesulfonic acid (TNBS), as described below.

2.3. Protein modification by trypsin

Enzymatic hydrolysis was performed with TBSP (12 g) dispersed with deionized water (120 ml) and adjusted to pH 7.0 with 0.1 M HCl or NaOH with stirring by a magnetic stirrer. The 10% w/v protein dispersion was transferred to a 500 ml flask to which was added 0.2% trypsin solution (24 mg/2 ml distilled water) to obtain an enzyme to substrate ratio of 1:50. The mixture was incubated at 55 °C in a shaking water bath for 1 or 24 h. Reaction progress, measured as the degree of hydrolysis (DH), was calculated from changes in fNH_2, as described by Adler-Nissen (1979);

$$DH(\%) = 100\left[\frac{[fNH_2]}{W^{*}A_a}\right] \quad (1)$$

where, W(g) is the weight of protein substrate (g), A_a (0.008 mol of peptide g^{-1} protein) is a constant and [fNH_2] is the concentration of free amine groups (mol g^{-1} protein), determined as described below.

2.4. Protein modification by pre-heating

TBSP (12 g) was dispersed with deionized water (120 ml) and adjusted to pH 7.0 with 0.1 M HCl or NaOH with agitation by a magnetic stirrer. The 10% w/v protein dispersion was transferred to a 500 ml flask and preheated at 55 °C for 1 h. The sample was then cooled over ice and refrigerated until used for analysis.

2.5. Determination of protein solubility

TBSP (40 mg) was suspended in 4 ml (w/w) of deionized water, adjusted to pH 2.0–9.0 using 0.1 M HCl or 0.1 M NaOH, and stirred by a magnetic stirrer for 60 min. Samples were centrifuged at 12,000*g* for 10 min, and the protein concentration of the supernatant was determined by the modified Lowry's method (Peterson, 1977). Protein solubility was expressed as percent of protein in the supernatant compared to the total crude protein ($N \times 6.25$), determined by the Kjeldahl method.

2.6. Analysis of free amino groups

Alterations in the concentration of free amino groups (fNH_2) were determined by reaction with trinitrobenzenesulfonic acid (TNBS), as described by Adler-Nissen (1979); 20 μl of protein sample (1.0 g l^{-1}) in phosphate buffer (pH 7) were added to 980 μl of 0.10 M sodium tetraborate buffer (pH 9.3) in a 1.5 ml cuvette, followed by 25 μl of 0.03 M TNBS. The mixture was agitated to ensure complete mixing, allowed to stand for 30 min at room temperature, and absorbance measurements recorded at 420 nm (A_{420}). The reagent blank was 25 μl of 0.03 M TNBS in 1 ml of 0.10 M sodium tetraborate buffer. fNH_2 was calculated from Eq. (2) where W(g) is the weight of protein per cuvette.

$$fNH_2\ (\text{moles/g}) = \frac{A_{420} \times 0.001025\ \text{l}}{20300\ (\text{l/mole cm}) \times 1\ \text{cm} \times W(\text{g})} \quad (2)$$

2.7. Determination of surface hydrophobicity

Surface hydrophobicity (S_0) was determined using l-anilino-8-naphthalene sulfonate (ANS) as a fluorescence probe, as described by Kato and Nakai (1980), with modifications by Alizadeh-Pasdar and Li-Chan (2000). Protein (1 g l^{-1} in 0.1 M phosphate buffer, pH 7.0) was serially diluted with buffer to obtain 20, 40, 60, 80 and 100 μg protein in a total volume of 2.8–3.00 ml (0.1 M phosphate buffer, pH 7.0). Then, 0.1 ml ANS (1.0 mM in 0.1 M phosphate buffer, pH 7.0) was added to a final concentration of 32 μM. After equilibration at room temperature for 15 min, ANS fluorescence intensity was measured with a spectrofluorimeter using an excitation wavelength of 380 nm and the emission wavelength setting at 475 nm. Bovine serum albumin was also analyzed in a similar manner as a standard. Surface hydrophobicity was calculated as the gradient of a graph of ANS fluorescence plotted against protein concentration. The resulting data were then normalized by the corresponding gradient for BSA, as shown by Alizadeh-Pasdar and Li-Chan (2000).

2.8. Emulsification characteristics

Emulsification characteristics were determined as described by Pearce and Kinsella (1978) with some modifications. Corn oil (10 ml) and protein solution (0.5% w/v, 30 ml) were homogenized with an Ultra Turrax® homogenizer (model T25 Basic IKA Labortechnik homogenizer, IKA® Works Inc., Wilmington, NC; Homogenizer 115 V, 50/60 Hz; dispersing tool: S25NK-19G, Fine, 19 mm. dia.) at a speed 22,000 rpm for 1 min at 23 ± 1 °C. Triplicate preparations of emulsion were made for each study.

Emulsification activity index (EAI) and emulsion stability index (ESI) for TBSP samples were determined by the turbidimetric technique of Pearce and Kinsella (1978). Aliquots (1 ml) of the emulsion were diluted serially by 1/50–1/500, using 0.1% sodium dodecyl sulphate (SDS). Absorbance readings were recorded at 500 nm (A_{500}) using disposable cuvettes (Fisher scientific, Chicago, IL) and a UV–Vis spectrophotometer (Thermo Spectronic, Rochester, NY). EAI was calculated from the relationships below;

$$\tau = \frac{2.303 \times A_{500} \times F}{l} \quad (3)$$

$$\text{EAI}\ (\text{m}^2/\text{g}) = \frac{2\tau}{\phi C} \quad (4)$$

where τ is turbidity, A is the sample absorbance, F is the sample dilution factor (50–500), l (0.01 m) is the light path length. The oil volume fraction for emulsions (ϕ) and the protein emulsifier concentration (C) were 0.25 and 5 kg m^{-3}, respectively.

Emulsion stability index (ESI) was determined by placing aliquots of emulsion (10 ml) inside 10.0-ml cylinders immediately after preparation. At 0 and 10 min following emulsion preparation, 0.1 ml samples were removed and diluted 50–500-fold prior to turbidity measurements. ESI was calculated from,

$$\text{ESI}\ (\text{min}) = \frac{\tau \times \Delta t}{\Delta \tau} \quad (5)$$

where $\Delta\tau$ is the change in turbidity after a time interval Δt (10 min) regardless of the sign.

2.9. Foaming capacity and foaming stability

Foaming capacity was determined, as described by Phillips et al. (1990), with modifications. Indices of foaming behaviour were calculated from,

$$\%\text{Overrun} = \frac{\text{Total volume} - \text{Drainage volume}}{\text{Initial protein volume}} \times 100 \quad (6)$$

$$\text{Foam stability} = \frac{\text{Initial protein volume} - \text{Drainage volume}}{\text{Initial protein volume}} \times 100 \quad (7)$$

2.10. Experimental design and statistical analysis

Investigations using the same batches of TBSP or modified TBSP were performed at least twice. Instrumental measurements were performed in triplicate. The properties of TBSP and 5-modified derivatives were compared using 1-way ANOVA. Multiple regression analysis of structure–functionality relationships was performed by fitting data to Eq. (8);

$$Y = m_1 S_0 + m_2 \text{Cp} + m_3 S_W + m_4 [\text{fNH}_2] + \text{constant} \quad (8)$$

where Y (emulsification activity index, emulsion stability index, foaming capacity or foaming stability) is the dependent variable. Modelling parameters (independent variables) were, surface hydrophobicity (S_0), sample crude protein (Cp), protein solubility in water (S_W) and free amino group concentration ([fNH_2]). A limited number of analysis were performed using a 2nd order equation to check for interactions: $Y = m_1 \ X_1 + m_2 \ X_2 + m_{1,2} X_1 X_2 + \text{constant}$. The coefficients for regression analysis (m_1, m_2, m_3, m_4) and t-values ($t = m_1/\text{SE}_1$) were employed as the basis for ANOVA with $p < 0.05$. Statistical analyses were performed by SPSS for Windows (V 11.50, SPSS Inc., Chicago, Illinois, USA), as described by Pallant (2001).

3. Results and discussion

3.1. Characteristics of modified threadfin bream sarcoplasmic protein

The characteristics of normal and modified threadfin bream sarcoplasmic proteins (TBSP) are summarized in Table 1. Protein modification produced TBSP derivatives with significant changes in surface hydrophobicity (S_0), free amino group (fNH_2) concentration and solubility (S_W) compared to the starting material. The order of S_0 values was, TBSP (preheat) < TBSP (acetylated) < TBSP < TBSP (succinylated) < TBSP (trypsin-24 h) < TBSP (trypsin-1 h). Past investigations show that acylation raises protein surface hydrophobicity (Knope et al., 1998; Krause, Mothes, & Schenke, 1996). Introducing an acyl group may also lead to protein dissociation and changes in tertiary structure (Gruener & Ismond, 1997a, 1997b), thereby exposing more hydrophobic sites to the ANS probe.

Transfer of ANS probe from an aqueous solution to a nonpolar protein site increases fluorescence quantum yield (Kato & Nakai, 1980; Stryer, 1965). A fluorescence increase also occurs when ANS is transferred from solution to a rotationally restricted site or when the ANS^- anion interacts with a positively charged protein (lysine ε-NH_2 and terminal NH_2) site (Nyman & Owusu-Apenten, 1997). Apparently, measuring surface hydrophobicity using the ANS fluorescence index may be subject to error, which has led some investigators to propose alternative fluorescence probes for the determination of S_0. Thus far, no single probe has been found to be completely satisfactory for all applications (Haskard & Li-Chan, 1998). The S_0 for TBSP was higher following trypsin treatment for 1 h than for 24 h, though values for fNH_2 and DH (%) were greater in the latter case.

Succinylated TBSP showed increased S_W, S_0 but decreased fNH_2 concentration compared to the unmodified TBSP (Table 1). Pre-treatment with trypsin at 55 °C increased the fNH_2 concentration from 318 to 594 μmol g^{-1} (DH = 2%) or 890 μmol g^{-1} (DH = 4.7%) after 1 h or 24 h, respectively. Compared to the unheated control, pre-heating TBSP at 55 °C for 1 h led to decreased S_W, S_0 and fNH_2.

The temperature treatment used in this study (with or without trypsin addition) was chosen to take account of two interesting features of TBSP. First, differential scanning calorimetry measurements showed that TBSP undergoes heat-denaturation with three peak transitions at 56, 66 and 76 °C (Krasaechol et al., 2005). The pre-heating temperature of 55 °C was just below the denaturation temperature for TBSP. Second, TBSP contains endogenous alkali proteases with optimal activity at 55–60 °C when assayed with casein (Krasaechol et al., 2005). Therefore,

Table 1
Characteristics of threadfin bream sarcoplasmic proteins and modified derivatives

Sample	Crude protein (%)	Free-NH_2 (μmol g^{-1})	Surface hydrophobicity (S_0)	Solubility (%)
TBSP (control)	42 (±0.31)^a	318 (±22)^a	1636 (±7.1)^a	45 (±0.6)^a
TBSP (succinylated)	59 (±0.9)^b	3.7 (±0.01)^b	1775 (±5.0)^b	60 (±0.2)^b
TBSP (acylated)	57 (±2.4)^b	−0.26 (±0.37)^b	1587 (±0.0)^c	19 (±0.4)^c
TBSP (trypsin, 1 h)	43 (±0.2)^a	594 (±8.4)^c	2487 (±6.4)^d	10 (±0.2)^d
TBSP (trypsin, 24 h)	43 (±0.2)^a	890 (±0.0)^d	1803 (±5.6)^b	4.7 (±0.4)^e
TBSP (preheat, 1 h)	44 (±2)^a	274 (±0.0)^e	1259 (±6.4)^e	8.0 (±0.11)^f

Surface hydrophobicity (S_0) scale is 1000 for bovine serum albumin, crude protein (Cp), solubility (S_W) and free amino group concentration ([fNH_2]). Data show mean values (±SD) for 6-replicates. Numbers in each column followed by a different letter are significantly different ($p < 0.05$).

incubation at 55 °C for 1 h was expected to either increase or decrease the solubility of TBSP as a consequence of thermal denaturation or enzymatic hydrolysis, respectively. Interestingly, the data show that proteolysis could be achieved (as judged by fNH_2 increase) though the partially hydrolyzed TBSP undergoes heat coagulation at 55 °C (Fig. 3).

3.2. Regression analysis and modelling structure–function relationships for TBSP

Modification of TBSP led to significant changes in functional properties as summarized in Table 2. Combining the data from Tables 1 and 2 should provide a basis for developing structure–activity relationships. However, it is not easy to establish the association between particular structural features (Table 1) and functional properties (Table 2) by inspection. One reason for difficulty is that protein characteristics may be interrelated, leading to confounding effects. Li-Chan, Nakai, and Wood (1984) employed principal component analysis to reduce the number of variables to key elements. Multiple regression analysis was then applied to ascertain quantitative structure–function relationships.

Since TBSP and the modified derivatives can be expected to conform to the same underlying biophysical principles, results from this study were pooled. The resulting data set was then subjected to multiple regression analysis. Table 3 shows the approach for model development, illustrated by fitting EAI (m^2/g) to Eq. (8). The adjusted squared regression coefficient (adjusted R^2) value for a 4-parameter equation (model 4) shows that this accounts for ~90% of the variation in EAI. Similarly, the 3-parameter model (produced by dropping the fNH_2 term from model 4) predicted 91% of the variations in EAI ($R^2 = 0.91$; F-value = 38, $p < 0.001$). Since F-values for models 3–4 were not significantly different ($p = 0.766$), the simpler model could be adopted to describe EAI (Table 3). It may be concluded that the fNH_2 term from Eq. (8) had no influence on the volume of emulsions produced using TBSP or modified TBSP.

Overall, between 86% and 99% of the variabilities in EAI, ESI, FC and FS were accounted for by four independent variables (S_0, Cp, S_W and fNH_2) in this study. The functional properties of TSBP could be modelled by the following equations: $EAI = 0.045S_0 + 2.845Cp + 1.388S_W - 200.7$ ($R^2 = 0.93$, $p < 0.001$); $ESI = -0.027S_0 - 15.302Cp + 1.9444S_W + 678.0$ ($R^2 = 0.977$, $p < 0.001$); $FC = 0.087S_0 + 2.732Cp - 0.05S_W + 10.567fNH2 - 109.159$ ($R^2 = 0.980$, $p < 0.001$); $FS = 0.038S_0 - 0.339S_W - 2.863fNH2 - 17.775$ ($R^2 = 0.907$, $p < 0.001$). Graphical presentations of selected TBSP functional properties modelled using the above equations, are shown in Figs. 1 and 2. All models are reasonably accurate when compared with actual observations. Thus the predicted values for FC were within the range 100–300%, compared to observed values of 142–277% (Table 2).

Table 3
Model development for fish protein emulsification activity index*

Model# (variables)	R^2 (adjusted)	F change	df1	df2	Sig. F change
1 (S_W, S_0)	0.767	19.150	2	9	0.001
2 (S_W, Cp)	0.772	19.572	2	9	0.001
3 (S_W, S_0, Cp)	0.910	37.984	3	8	0.000
4 (S_W, S_0, Cp, [fNH_2])	0.898	0.096	1	7	0.766

* Data were fitted with Eq. (8). Models #1–4 examined the extent to which emulsification activity index is explained by, protein solubility in water (S_W), surface hydrophobicity (S_0), crude protein content (Cp) and free amino group concentration (fNH_2).

Additional structure – function trends may be discerned from regression parameters shown in Table 4. Large magnitude t-values ($t = m_1/SE_1$) indicate which variables have the most impact on functional properties. For instance, values for t-value show that S_W has a positive influence on protein emulsification (ESI and EAI) but a negative influence on foaming characteristics (FC, FS). From the magnitude of the respective t-values, S_0 had a greater influence on FC and FS than on emulsification (Table 4). Interestingly also, the fNH_2 term did not affect EAI and ESI, as described previously in relation to model 3 (Table 3). In contrast, foaming characteristics were significantly affected by fNH_2.

3.3. Observed characteristics of modified protein preparations

Some tentative structure–function relationships for TBSP and five modified TBSP derivatives are discussed above, without detailed references to particular forms

Table 2
Functionality of threadfin bream sarcoplasmic proteins and modified derivatives

Sample	EAI (m^2/g)	ESI (min)	FC (%)	FS (%)
TBSP (control)	46 (±3.5)[a]	63 (±1.8)[a]	165 (±1.41)[a]	26.0 (±0.0)[a]
TBSP (succinylated)	145 (±2.8)[b]	160 (±1.4)[b]	175 (±1.41)[b]	26 (±2.8)[a]
TBSP (acylated)	35 (±1.4)[c]	197 (±5.3)[c]	176 (±0.0)[b]	37 (±1.41)[b]
TBSP (trypsin, 1 h)	14 (±6.93)[e]	19 (±5.1)[d]	277 (±4.24)[c]	57 (±1.4)c
TBSP (trypsin, 24 h)	6 (±0.71)[d]	12 (±11)[d]	257 (±0.0)[d]	22.5 (±0.0)[a]
TBSP (preheat, 1 h)	14 (±1.4)[e]	19 (±0.7)[d]	142 (±2.8)[e]	19.0 (±1.34)[a,d]

Emulsification activity index (EAI), emulsion stability index (ESI), foaming capacity (FC) and foaming stability (FS). Data show mean values (±SD) for 6-replicates. Numbers in each column followed by a different letter are significantly different ($p < 0.05$).

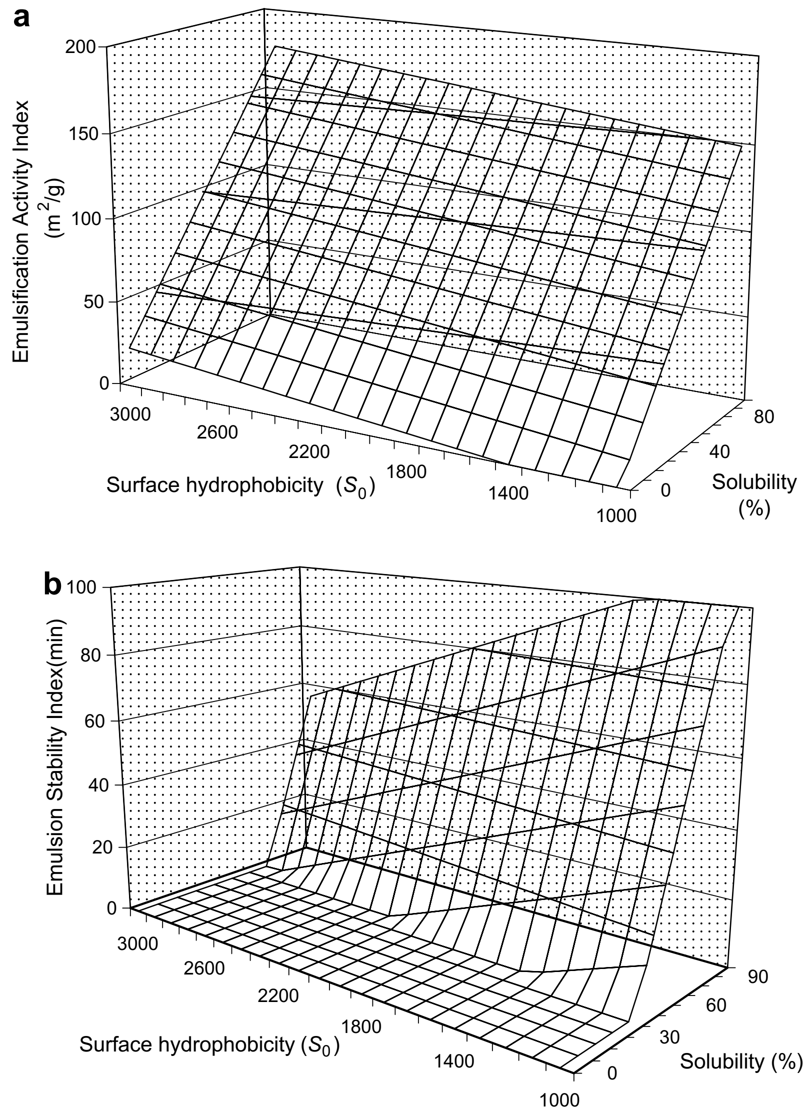

Fig. 1. Effect of surface hydrophobicity (S_0) and protein solubility on the (a) emulsification activity index or (b) stability of emulsions produced with threadfin bream sarcoplasmic protein (TBSP), succinylated TBSP, acylated TBSP, trypsin hydrolyzed TBSP or TBSP preheated at 55 °C (1 h). Graph shows predicted responses giving the best best-fit using pooled results for all proteins (see text for details).

of protein modification. The following sections consider functional properties in greater detail and how each may be affected by different types of protein modification.

The solubility of unmodified TBSP was 48% at pH 2–4 and pH 7–9 with minimum solubility at around pH 5 (Fig. 3a). Within the range pH 5–9, succinylation increased the solubility of TBSP. There was a shift in the pH minimum for solubility toward low pH values after succinylation or acetylation. Therefore, acylated TBSP may be unsuitable for applications in acidic foods. Such results agree with prior investigations which showed that acetylation of bean leguminin decreased its solubility at low pH (Knope et al., 1998).

Hydrolysis by trypsin (1 h) increased TBSP solubility by a maximum of 200%, depending on the solvent pH (Fig. 3b). Protein hydrolysis was performed at 55 °C to take advantage of the presence of thermostable proteases in threadfin bream muscle. (Krasaechol et al., unpublished results). However, there was little advantage to be gained from this practice. Low temperature enzymatic hydrolysis is recommended to reduce heating costs. Food proteins are routinely hydrolyzed to increase their solubility (Kristinsson & Rasco, 2000).

3.4. Improving the emulsification properties of TBSP by succinylation

The EAI of TBSP (succinylated) was significantly higher than those of any other modified TBSP (Table 2). Based on the structure–function analysis presented earlier, the high EAI value for TBSP (succinylated) is likely to arise from changes in protein S_W and S_0. Succinylation converts protein cationic fNH_2 to anionic succinate residues. The

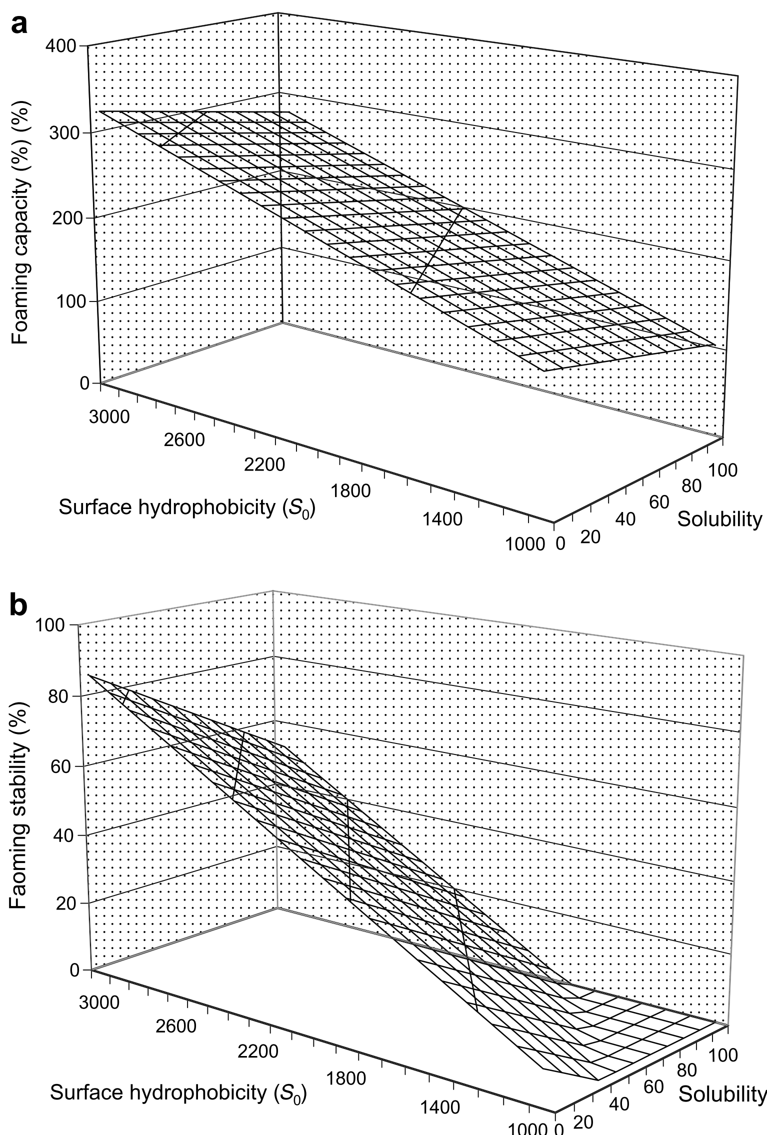

Fig. 2. Effect of surface hydrophobicity (S_0) Creative fluorescence and protein solubility % on the (a) foaming capacity and (b) foaming stability. Foams were prepared using threadfin bream sarcoplasmic protein (TBSP), succinylated TBSP, acylated TBSP, trypsin hydrolyzed TBSP or TBSP preheated at 55 °C (1 h). Graph shows predicted responses giving the best best-fit using pooled results for all proteins (see text for details).

Table 4
Effect of different variables on threadfin bream sarcoplasmic protein functionality

Variable	t-value[a]			
	EAI	ESI	FC	FS
Constant	-4.76^{*}	13.71^{***}	-3.19^{*}	-2.93^{*}
Surface hydrophobicity (S_0)	3.85^{**}	-0.11	10.10^{***}	10.30^{***}
Crude protein (Cp)	3.90^{**}	-17.83^{***}	4.30^{**}	ns
Water solubility (S_W)	5.71^{**}	6.79^{***}	-2.95^{*}	-4.57^{**}
Free amino groups (fNH_2)	ns	ns	6.35^{***}	-5.30^{***}
R^2 (adjusted)	0.910	0.970	0.969	0.910
$F_{4,7}$	38.0^{***}	113.6^{***}	86.3^{***}	37.0^{***}

Levels of statistical significance are $p \leqslant 0.05$ (*), $p \leqslant 0.01$ (**), or $p \leqslant 0.001$ (***) or non-significant (ns) $p > 0.05$.

[a] *Notes*: A large magnitude of t-value ($t = m_1/SE_1$) indicates greater influence on emulsification activity index (EAI), emulsification stability index (ESI), foaming capacity (FC) or foam stability (FS).

increase in the number of negative charges enhances protein solubility under neutral or alkaline solvent conditions. The introduction of the 4-carbon succinate residue also increases S_0. Raising the number of charged and nonpolar residues simultaneously would increase the amphipathic character of protein molecules, leading to enhanced EAI

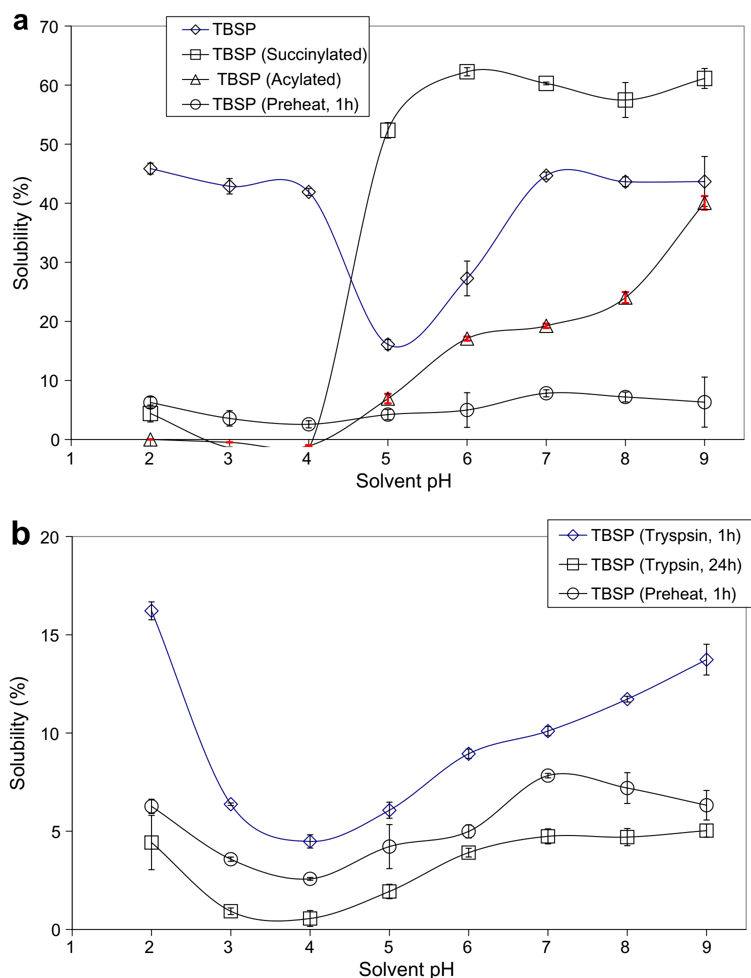

Fig. 3. Effect of pH on the solubility of threadfin bream sarcoplasmic protein (TBSP) and modified TBSP without (a) and with heat pre-treatment (b) (see text for details).

(Habeeb, Cassidy, & Singer, 1958). With soy protein isolate (SPI), electrostatic attractions between neighbouring fNH_2^+ and carboxyl groups is believed to enhance protein–protein interactions, leading to low solubility. By contrast, electrostatic repulsions between newly introduced carboxylate groups reduce protein–protein interactions whilst strengthening protein–water interactions. For SPI derivatized with 0.5 and 2 g of succinic anhydride, S_W increased to a similar degree. Exhaustive succinylation was not required to produce a substantial increase in protein solubility (Franzen & Kinsella, 1976).

Table 4 shows that structural requirements for a high ESI were different from those described for a high EAI. Emulsion stability was dependent on S_W and was not hugely affected by S_0. ESI is also likely to be affected by solvent viscosity which was not measured in this study (Table 4).

3.5. Improving the foaming capacity of TBSP by trypsin hydrolysis

TBSP (Trypsin, 1 h) had the highest foam capacity of all the derivatives tested (Table 2). For all TBSP samples considered together, foamability increased with increasing S_0 and fNH_2 (Tables 1 and 4). Though enzyme treatment led to an increase in S_W, this parameter was not associated with an increase in FC. The value for fNH_2 obtained for TBSP was ~3.2 ± × 10^{-4} mol g^{-1} before trypsin modification which is 58% of the total lysine residues determined by amino acid analysis (Krasaechol et al., 2005). Significant numbers of lysine residues for TBSP were not solvent-accessible, probably because of the low (46%) solubility of the starting ingredient. The fNH_2 concentration increased by 90% following hydrolysis by trypsin. Acetylation, succinylation or pre-heating decreased fNH_2 concentration by 98%, 100% or 9%, respectively. Whereas an increase in fNH_2 contributed positively to foam volume, it contributed negatively to foam stability (Table 4).

The stabilities of protein foams produced using TBSP (Trypsin 1 h or 24 h) were lower than those from TBSP, TBSP (succinylated) or TBSP (acylated), probably because FC and FS are dependent on different protein molecular characteristics. To generate a high foam volume requires rapid adsorption of protein and peptides at the air–water interface during whipping or bubbling, and ability to undergo rapid conformational rearrangement. By comparison, a high FS requires a thick, elastic, cohesive, continu-

ous, air-impermeable protein film around each gas bubble (Zayas, 1997). A decrease in protein molecular weight and solvent viscosity arising from protein hydrolysis will also undermine foam stability.

Past investigations show that protein succinylation can decrease both FC and FS (Phillips & Kinsella, 1990). This was explained in terms of decreased surface pressure development at the air–water interface, due to charge–charge repulsion between succinate groups introduced into the β-lactoglobulin molecule. By contrast, partial (27.5%) succinylation improved foaming characteristics because, where only a proportion of fNH_2 are modified, the capacity for electrostatic attractions remains leading to the formation of cohesive interfacial films (Phillips & Kinsella, 1990).

4. Conclusions

The results of this study suggest that the functional properties of fish wash-water proteins, modelled by TBSP, could be extended by protein modification. However, emulsification and foaming characteristics were differentially affected by, protein surface hydrophobicity, solubility, and the concentration of free amino groups. Protein emulsification capacity was enhanced by succinylation, probably because of the increase in S_W and S_0. In contrast, emulsion stability was positively affected by increasing S_W and not S_0. Therefore, the functional properties of TBSP could be modified by succinylation and trypsin hydrolysis to increase emulsification and foaming capacity, respectively. The requirements for emulsification and foaming appear to be different from those needed for stabilizing the resulting dispersions. Polysaccharide thickening agents could be examined as secondary stabilizers for fish-protein stabilized emulsions and foams. Additional studies would be needed to establish whether the structure–functionality relationships revealed in this study also apply to other food protein ingredients.

Acknowledgement

The authors are grateful for financial support to NK from the Government of Thailand.

References

Achouri, A., Wang, Z., & Xu, S. Y. (1998). Enzymatic hydrolysis of soybean protein isolate and effect of succinylation on the functional properties of resulting protein hydrolysate. *Food Research International, 21*(9), 617–623.

Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry, 27*, 1256–1262.

Alizadeh-Pasdar, N., & Li-Chan, E. C. Y. (2000). Comparison of protein surface hydrophobicity measured at various pH values using three different fluorescent probes. *Journal of Agricultural and Food Chemistry, 48*, 328–334.

Ashi, I. N. A., Simpson, B. K., & Smith, J. P. (1996). Mechanism for controlling enzymatic reactions in foods. *Critical Reviews in Food Science & Nutrition, 36*(1–2), 1–30.

Benjakul, S., Morrissey, M. T., Seymour, T. A., & An, H. (1997). Recovery of proteinase from Pacific whiting surimi wash water. *Journal of Food Biochemistry, 21*, 431–443.

Benjakul, S., Seymour, T. A., Morrissey, M. T., & An, H. (1998). Characterization of proteinase recovered from Pacific whiting surimi wash water. *Journal of Food Biochemistry, 22*, 1–16.

Campbell, L., Raikos, V., & Euston, S. R. (2003). Modification of functional properties of egg-white proteins. *Nahrung, 47*, 369–376.

Davis, P. J., & Williams, S. C. (1998). Protein modification by thermal processing. *Allergy, 53*(Suppl. 46), 102–105.

Djagny, K. B., Wang, Z., & Xu, S. Y. (2001). Gelatin: A valuable protein for food and pharmaceutical industries. *Critical Reviews in Food Science and Nutrition, 41*, 481–492.

Fares, K., Landy, P., Guilard, R., & Voilley, R. (1998). Physicochemical interactions between aroma compounds and milk proteins: Effect of water and protein modification. *Journal of Dairy Science, 81*, 82–91.

Franzen, K. L., & Kinsella, J. E. (1976). Functional properties of succinylated and acetylated leaf protein. *Journal of Agricultural and Food Chemistry, 24*, 914–919.

Gruener, L., & Ismond, M. A. H. (1997a). Effects of acetylation and succinylation on the physicochemical properties of the canola 12S globulin: Part I. *Food Chemistry, 60*, 357–363.

Gruener, L., & Ismond, M. A. H. (1997b). Effects of acetylation and succinylation on the functional properties of the canola 12S globulin. *Food Chemistry, 60*, 513–520.

Guan, X., Yao, H. Y., Chen, Z. Z., Zhang, M. D., et al. (2007). Some functional properties of oat bran protein concentrate modified by trypsin. *Food Chemistry, 101*, 163–170.

Habeeb, A. F. S. A., Cassidy, H. G., & Singer, S. J. (1958). Molecular structural effects produced in proteins by reaction with succinic anhydride. *Biochimica et Biophisica Acta, 29*, 589–593.

Haskard, C. A., & Li-Chan, E. C. Y. (1998). Hydrophobicity of bovine serum albumin and ovalbumin determined using uncharged (PRODAN) and anionic (ANS-) fluorescence probes. *Journal of Agricultural and Food Chemistry, 46*, 2671–2677.

Hamada, J. S. (1994). Deamidation of food proteins to improve functionality. *Critical Reviews in Food Science and Nutrition, 3*, 283–292.

Jaoen, P., & Quemeneur, F. (1992). Membrane filtration for waste-water protein recovery. In G. M. Hall (Ed.), *Fish processing technology* (pp. 212–248). Glasgow: Blackie.

Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochimica et Biophysica. Acta, 624*, 13–20.

Kawai, Y., Ohno, R., Inoue, N., & Shinano, H. (1995). Emulsifying activity of heat treated sarcoplasmic protein from sardine. *Fisheries Science, 61*, 852–855.

Kim, J. S., & Park, J. W. (2004). Characterisation of acid-soluble collagen from Pacific whiting surimi processing by products. *Journal of Food Science, 69*, C637–C642.

Kinsella, J. E. (1976). Functional properties of proteins in foods: A survey. *Critical Reviews of Food Science and Nutrition, 8*, 219–280.

Knope, C., Schwenke, K. D., Mothes, R., Mothes, R., Mikheeva, L. M., Grinberg, V. Y., et al. (1998). Acetylation and succinylation of faba bean legumin: Modification of hydrophobicity and conformation. *Nahrung, 42*, 194–196.

Kosters, H. A., Broersen, K., de Groot, J., Simons, J. W. F. A., Wierenga, P., & de Jongh, H. H. J. (2003). Chemical processing as a tool to generate ovalbumin variants with changed stability. *Biotechnology and Bioengineering, 84*, 61–70.

Krasaechol, N., Sanguandeekul, R., Duangmal, K., & Owusu-Apenten, R., (2005). Characteristics and functional properties of sarcoplasmic proteins from threadfin bream (*Nemipterus hexodon*). *IFT Annual Meeting Abstract* 71B–14.

Krause, J. P., Mothes, R., & Schenke, K. D. (1996). Some physicochemical and interfacial properties of native and acylated legumin from faba beans (*Vicia faba* L). *Journal of Agricultural and Food Chemistry, 44*, 429–437.

Kristinsson, H. G., & Rasco, B. A. (2000). Fish protein hydrolysates: Production, biochemical, and functional properties. *Critical Reviews in Food Science and Nutrition, 40*, 43–81.

Lakkis, J., & Villota, R. (1992). Comparative performance of chemically and enzymatically modified whey proteins. *Food Chemistry, 43*, 93–105.

Li-Chan, E., Nakai, S., & Wood, D. F. (1984). Hydrophobicity and solubility of meat proteins and their relationship to emulsifying properties. *Journal of Food Science, 49*, 345–350.

Lin, T. M., Park, J. W., & Morrissey, M. T. (1995). Recovered protein and reconditioned water from surimi processing waste. *Journal of Food Science, 60*, 4–9.

Marti, C., Roeckel, M., Aspe, E., & Kanda, H. (1994). Recovery of proteins from fishmeal factory wastewaters. *Process Biochemistry, 29*, 39–46.

Montero, P., & Gomez-Guillen, M. C. (1998). Recovery and functionality of wash water protein from krill processing. *Journal of Agricultural and Food Chemistry, 46*, 3300–3304.

Morioka, L., Nishimura, T., Obatake, A., & Shimizu, Y. (1997). Relationship between the myofibrillar protein gel strengthening effect and the composition of sarcoplasmic proteins from Pacific mackerel. *Fisheries Science, 63*, 111–114.

Morrisey, M. T., & Tan, S. M. (2000). World resources for surimi. In J. W. Park (Ed.), *Surimi and surimi seafood* (pp. 1–21). New York: Marcel Dekker Inc.

Morrisey, M. T., Lin, J., & Ismond, A. (2005). Waste management and by-product utilization. In J. W. Park (Ed.), *Surimi and surimi seafood* (second ed., pp. 279–323). New York: Marcel Dekker Inc.

Nyman, R., & Owusu-Apenten, R. K. (1997). The effect of heat treatment on anilinonaphthalene-8-sulfonate binding to rapeseed albumin (napins). *Journal of the Science of Food and Agriculture, 74*, 485–489.

Oliver, C. M., Melton, L. D., & Stanley, R. A. (2006). Creating proteins with novel functionality via the Maillard Reaction: A review. *Critical Reviews in Food Science & Nutrition, 46*, 337–350.

Owusu-Apenten, R. K. (2004). Testing protein functionality. In R. Yada (Ed.), *Proteins in food processing* (pp. 217–244). New York: Woodhead Pub.

Pallant, J. (2001). *SPSS survival manual*. Maidenhead: Open University Press.

Panyam, D., & Kilara, A. (1996). Enhancing the functionality of food proteins by enzymatic modification. *Trends in Food Science & Technology, 7*(4), 120–125.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry, 26*, 716–723.

Peterson, G. L. (1977). A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Analytical Biochemistry, 83*, 346–356.

Phillips, L. G., German, J. B., O'Neil, T. E., Foegeding, E. A., Harwalkar, V. R., Kilara, A., et al. (1990). Standardized procedure for measuring foaming properties of three proteins, a collaborative study. *Journal of Food Science, 55*, 1441–1453.

Phillips, L. G., & Kinsella, J. E. (1990). Effects of Succinylation on β-lactoglobulin foaming properties. *Journal of Food Science, 55*, 1735–1739.

Roller, S., & Dea, I. C. M. (1992). Biotechnology in the production and modification of biopolymers for foods. *Critical Reviews in Biotechnology, 12*(3), 261–277.

Sathivel, S., Bechtel, P. J., Babbit, J., Prinyawiwatkul, W., Negulescu, I. I., & Reppond, K. D. (2004). Properties of protein powders from arrowtooth flounder (*Atheresthes stomias*) and herring (*Clupea harengus*) by-products. *Journal of Agricultural and Food Chemistry, 52*, 5040–5046.

Sathivel, S., Bechtel, P. J., Babbitt, J. K., Prinyawiwatkul, W., & Patterson, M. (2005). Functional, nutritional and rheological properties of protein powders from arrowtooth flounder and their application in mayonnaise. *Journal of Food Science, 70*, E57–E63.

Scilingo, A. A., Ortiz, S. E. M., Martinez, E. N., & Anon, M. C. (2002). Amaranth protein isolates modified by hydrolytic and thermal treatments. Relationship between structure and solubility. *Food Research International, 35*(9), 855–862.

Sikorski, Z. E., & Naczk, M. (1981). Modification of technological properties of fish protein concentrate. *Critical Reviews in Food Science and Nutrition, 14*, 201–230.

Stryer, L. (1965). The interaction of a naphthalene dye with apomyoglobin and apohemoglobin. A fluorescent probe of nonpolar binding sites. *Journal Molecular Biology, 13*, 482–495.

Torres, J. A., & Savant, V. D. (2001). Recovery of low concentrations of proteins by chitosan–polyanion complexes: Applications to waste water treatments. *Proceedings of the 8th International Congress on Engineering and Food (ICEF 8), Puebla City, April 2000* (Vol. 2, pp. 1925–1929). PA: Technomic Publishers, Lancaster.

Vojdany, F., & Whitaker, J. R. (1994). Chemical and enzymatic modification of proteins for improved functionality. In N. S. Hettiarachchgy & G. R. Ziegler (Eds.), *Protein functionality in food systems* (pp. 261–309). New York: Marcel Dekker Inc..

Wibowo, S., Savant, V., Cherian, G., Savage, T. F., & Torres, J. A. (2005). Evaluation as a feed ingredient of surimi wash water protein recovered using a chitosan–alginate complex. *Journal of Aquatic Food Product Technology, 14*, 55–72.

Wu, W. U., Hattiarachy, N. S., & Qi, M. (1998). Hydrophobicity, solubility, and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. *Journal of the American Oil Chemists Society, 75*, 845–850.

Zayas, J. F. (1997). *Functionality of proteins in food*. New York: Springer (p. 373).