Food Sci. Biotechnol. 25(6): 1569-1575 (2016)
DOI 10.1007/s10068-016-0242-0

Food Science
and
Biotechnology

Protein functionality of concentrates prepared from yellowfin tuna (*Thunnus albacares*) roe by cook-dried process

Sung Hwan Park, Hyun Ji Lee, In Seong Yoon, Gyoon-Woo Lee, Jin-Soo Kim[1], and Min Soo Heu*

Department of Food and Nutrition/Institute of Marine Industry, Gyeongsang National University, Jinju, Gyeongnam 52828, Korea
[1]Department of Seafood Science and Technology/Institute of Marine Industry, Gyeongsang National University, Tongyeong, Gyeongnam 53064, Korea

Received July 11, 2016
Revised October 5, 2016
Accepted October 13, 2016
Published online December 31, 2016

*Corresponding Author
Tel: +82-55-772-1440
Fax: +82-55-772-1430
E-mail: minsheu@gnu.ac.kr

pISSN 1226-7708
eISSN 2092-6456

Abstract Three kinds of roe protein concentrates (RPCs: boil-dried concentrate, BDC; steam-dried concentrate, SDC; freeze-dried concentrate, FDC) were prepared from yellowfin tuna to produce value added products for food applications. The buffer capacities of the RPCs were higher under alkaline than under acidic conditions. The water holding capacities of the RPCs were in range 4.5–4.7 g/g protein at pH 6.0. The protein solubility of the FDC (14.2%) was higher than those of the BDC (5.4%) and SDC (5.5%) at pH 6.0. The foaming capacity of the FDC (156.8%) was higher than those of the BDC (109.7%) and SDC (109.4%); the FDC foam was stable for 60 min. The oil-in-water emulsifying activity index of the FDC (12.2 m^2/g protein) exceeded those of the BDC and SDC (2.2 m^2/g protein). Protein concentrates from yellowfin tuna roe may be useful as a potential protein source and as a high-value food ingredient.

Keywords: roe concentrate, yellowfin tuna, protein functionality, cook-dried process

Introduction

The yellowfin tuna (*Thunnus albacares*) is an epipelagic marine fish that inhabits the mixed surface water layer above the thermocline. The Korean fisheries catch was 44,013 tons in 2013 (1). Most yellowfin meat is canned; the fish is also widely consumed raw. During the fish dressing procedure, heads, scales, skin, fat, viscera and roe are mostly treated as solid waste. As much as 70% of the original raw carcass is discarded (2). Only a small proportion of the fish dressing byproducts is processed into value-added products for either industrial application or animal/human consumption (3). Byproduct utilization will certainly improve the economic prospects of the processing industry, enhance the nutritional benets of fishery products and reduce waste discards or treatment (4). Considerable attention is now focused on ways to convert seafood byproducts into valuable functional ingredients, such as bioactive peptides (5), chitosan (6), and chitooligosaccharides (COS) (7).

Roe is high valuable seafood resource, and large quantities produced at the spawning season (8). Individual yellowfin tuna produce, on average, 2–3 million eggs in their roe. The roe, which makes up 1–3% of fish body weight, is generally useful to pet food preparation and agricultural animal feed or human consumption. Albumins, ovoglobulin, and collagen make up 11, 75, and 13% of roe mass, respectively (9), and roe has high essential amino acid contents. A processing procedure is demanded to convert under-utilized yellowfin tuna roe into a more acceptable and value-added forms as roe protein concentrate. Protein concentrates are widely useful to food ingredients in the seafood and food manufacture because of their high nutritional value, food functionalities, high essential amino acids contents, and protein level, but low contents of anti-nutritional factors (10). Fish protein concentrates in powder form have profits because powders do not demand specific keeping conditions and can readily be incorporated as food ingredients (11).

Drying preserves fish by inactivating hydrolases and removing the moisture needs for mold and bacterial growth (12-14). Changes caused by drying may have either beneficial or detrimental effects on the nutritional and functional characteristics of a processed product (15). MacDonald *et al*. (16) reported on a range of different drying methods for preparing their products. Sathivel *et al*. (17) showed that the dried protein powders of arrowtooth flounder muscle and herring head have many desirable functional protein components. The food functionalities of a freeze-dried protein powder from herring byproducts enables its use as gelling and emulsifier agents that competes commercially with soy protein and egg albumin powders (17). Galla *et al*. (4) described amino acid compositions and their physico-chemical characteristics and food functionalities and antioxidative activities of roe protein concentrates prepared from *Channa striatus* and *Lates calcarifer*. Sathivel and Bechtel (18)

examined the soluble protein powders produced from Alaska pollack (*Theragra chalcogramma* byproducts and their properties. Chalamaiah *et al*. (19) described the physico-chemical and nutritional properties of mrigal (*Cirrhinus mrigala)* roe protein concentrates and their practical use in pasta. Boiling and steaming fish before the drying process improves digestibility, enhances palatability and increases food safety by perishing harmful bacteria, other parasites, and microorganisms.

In our previous study, we prepared concentrates from yellowfin tuna roe and confirmed the proximate compositions and mineral and amino acid contents of concentrates (20). The current study focused on the food functionalities of the concentrates measured as buffering capacity, water holding capacity, solubility, foaming capacity, and emulsifying capacity and stability for use as functional ingredients and protein supplements.

Materials and Methods

Material Yellowfin tuna (*Thunnus albacares*) roe was obtained from Dongwon F&B Co., Ltd. (Changwon, Korea). Roe was stored at –70°C in sealed polyethylene bags and transferred to the laboratory. Frozen roe was partially thawed for 24 h at 4°C, cut into small pieces *ca*. 1.5–3.0 cm thick and then minced in a food grinder (SFM-555SP; Shinil Industrial Co., Ltd., Seoul, Korea). Minced roe was frozen at –20°C until used.

Chemicals Sodium dodecyl sulfate (SDS) was purchased from Bio Basic, Inc., (Markham, Canada). Bovine serum albumin (BSA), egg white, sodium carbonate, sodium hydroxide, sodium L-tartrate, and potassium hydroxide were obtained from the Sigma-Aldrich Co. (St. Louis, MO, USA). Copper (II) sulfate pentahydrate was purchased from Yakuri Pure Chemicals Co., Ltd. (Kyoto, Japan). Folin-Ciocalteu reagent was purchased from the Junsei Chemical Co., Ltd. (Tokyo, Japan). Soybean oil was purchased from Ottogi Co., Ltd. (Seoul, Korea). All reagents used were analytical grade.

Preparation of roe protein concentrates (RPCs) RPCs were prepared using a previously described procedure (20). Briefly, a freeze-dried concentrate (FDC) was prepared (without prior heating) in a freeze dryer (PVTFD50A; ILSHINBIOBASE Co., Ltd., Dongducheon, Korea) for use as a sample control. To prepare a boil-dried roe concentrate (BDC), a sample immersed in five volumes of distilled deionized water (DDW) was boiled for 20 min after the sample core temperature had reached 80°C. A steam-dried roe concentrate (SDC) was prepared by steaming for 20 min after the core temperature had reached 80°C. Cooked (boiled or steamed) samples were dried at 70±1°C for 15 h in an incubator (VS-1203P3V; Vision Scientific, Co., Ltd., Daejeon, Korea). Boiled and steam-dried samples were ground to powder in a food grinder and passed through 180 mesh. Ground powders produced by these two processes were identified as BDC and SDC, respectively.

Buffer capacity Buffer capacity was measured using a procedure described by Galla and Dubasi (21) with slight modifications. Briefly, 300 mg of sample was dispersed in 30 mL DDW to which we added measured volumes of 0.5 M NaOH or HCl in small increments; the corresponding changes in pH (in both alkaline and acidic ranges) were noted. The volumes of alkali and acid added were plotted against pH and the buffer capacity of the sample in each range was expressed as the mean molarity (mM) of NaOH or HCl per gram of protein required to bring about a change of one pH unit.

Water holding capacity The water holding capacity (WHC) of each sample was measured following the method of Shahidi *et al*. (22) with minor modifications. Each sample (300 mg) was dispersed in 30 mL of DDW and stirred with a magnetic stirrer at room temperature for 1 h. The mixture was centrifuged at 12,000×*g* for 20 min at 4°C. WHC was determined from the difference in weights and was expressed as the weight of water absorbed per gram of protein:

$$\text{WHC (g/g protein)} = \frac{\text{Weight of pellet (g)} - \text{Weight of sample (g)}}{\text{Weight of sample (g)} \times C}$$

where C is protein concentrate (%).

Protein solubility To determine protein solubility, we dispersed each 300 mg sample in 30 mL DDW and adjusted the pH values of the mixtures to 2, 4, 6, 7, 8, 10, and 12 with either 0.5 N HCl or 0.5 N NaOH. The mixtures were stabilized at room temperature for 30 min prior to centrifugation at 12,000×*g* for 20 min. The protein content in the supernatant was determined with Lowry's method (23) using bovine serum albumin as the protein standard. The total protein content in each 20 mg sample was determined using the Lowry method after solubilization in 0.5 N NaOH. Protein solubility was calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100$$

Foaming capacity and foam stability The foaming capacity (FC) and foam stability (FS) of sample solutions (1%, w/v) were determined following the method of Thiansilakul *et al*. (24) with slight modifications. We transferred 10 mL of 1% (w/v) sample solution into a 25 mL volumetric cylinder. The solution was homogenized in a Polytron® PT 1200E device (Kinematica AG, Lucerne, Switzerland) at 12,500 rpm for 1 min at room temperature. The samples were allowed to stand for 1, 15, 30, or 60 min. The FCs and FSs were then calculated from the following equations:

$$\text{Foaming capacity (\%)} = VT/V_0 \times 100$$
$$\text{Foam stability (\%)} = (Ft/Vt)/(VT/V_0) \times 100$$

where VT is total volume after whipping, V_0 is the original total volume before whipping, Ft and Vt are total foam and total volume, respectively, after samples had been standing at room temperature

for different periods of time (t=15, 30, and 60 min).

Oil-in-water emulsifying activity index and emulsion stability index The emulsifying activity index (EAI) and emulsion stability index (ESI) were determined following the procedures of Pearce and Kinsella (25) with slight modifications. Soybean oil was combined with 1% (w/v) samples in a 1:3 (v/v) mixture; the mixtures were homogenized in a Polytron® PT 1200E device at 12,500 rpm for 1 min. A 50 μL aliquot of the emulsion was pipetted from the bottom of a volumetric cylinder immediately after homogenization and 10 min later and then mixed with 5 mL of 0.1% sodium dodecyl sulfate (SDS) solution. The absorbance of the mixture was measured at 500 nm (UV-2900; Hitachi, Kyoto, Japan).

We used the absorbance measured immediately after emulsion formation ($A_{0\,min}$) and 10 min later ($A_{10\,min}$) to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI) as follows:

$$EAI\ (m^2/g)=\frac{2\times 2.303\times A\times D}{l\times\phi\times C}$$

where *A* is absorbance at 500 nm, *D* is a dilution factor (100), *l* is the path length of the cuvette (1 cm), ϕ is oil volume fraction (0.25) and *C* is the protein concentration in the aqueous phase (g/mL);

$$ESI\ (min)=\frac{A_0\times\Delta t}{\Delta A}$$

where $\Delta A = A_0 - A_{10}$, Δt=10 min, A_0 and A_{10} are absorbance measured at the beginning and 10 min later, respectively.

Statistical analysis Each measurement was replicated at least three times. Values are expressed as mean±SD. Significant effects were detected by analysis of variance (ANOVA) using SPSS 12.0 KO software (SPSS Inc., Chicago, IL, USA) for Windows. Multiple means comparisons were performed with the Duncan's multiple range test. Statistically significant differences were identified when $p<0.05$.

Results and Discussion

Buffer capacity The initial pH values and buffer capacities of the RPCs are depicted in Fig. 1. Dispersed RPCs (1%, w/v) in DDW had initial pH values of 5.9, 6.1, and 6.1 in the FDC, BDC, and SDC preparations, respectively. These values were lower than that of egg white (pH 7.4). A greater RPC buffer capacity was determined in alkali than in acid, indicating that larger volumes of alkali were required to change the pH. The FDC samples had higher buffer capacity in alkaline medium than the BDC, SDC, and egg white samples had ($p<0.05$). A pH change from 5.9 to 12 required 295.5 mM of NaOH/g of FDC protein. A similar buffer capacity trend was reported for roe protein concentrates obtained from *Channa striatus* and *Lates calcarifer* (4). During the preparation of protein isolates from the defatted seed flour of *Sterculia urens*, 0.44 mM (NaOH/g) was required to change the pH from 6.83 to 10 (21). Buffer capacity of tuna roe concentrates is different from other protein concentrates. The reason why has different isoelectric point and protein origin. These data contribute to the design of procedures for industrial-scale preparations of protein hydrolysates or isolates (4).

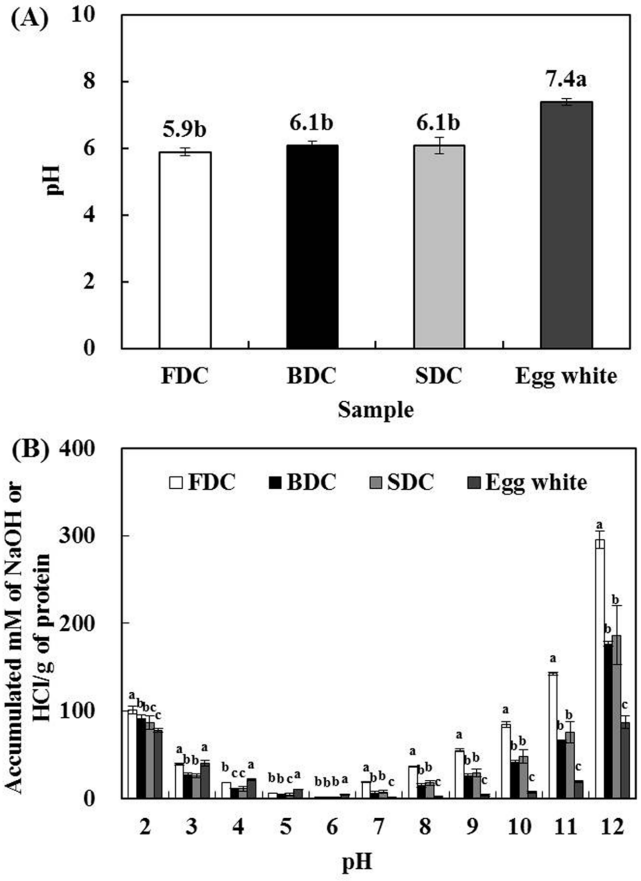

Fig. 1. pH values (A) and buffer capacity (B) of roe protein concentrates (RPCs) from yellowfin tuna prepared by cook-dried process. Data are mean±standard deviation of triplicate determinations. Values with different letter within samples are significantly different at $p<0.05$ by Duncan's multiple range test.

Water holding capacity Water holding capacity (WHC) is a protein functionality related to hydration. Mohamed *et al*. (26) reported that interactions among water, oil, and proteins are very important in food systems because of their effects on flavor and texture. Thus, we analyzed the WHC (Fig. 2A) of the RPCs and egg white (as a positive control) through pH shifts ranging from 2.0 to 12.0 and under conditions of stable pH. The WHCs of the FDC, BDC, SDC, and egg white samples under stable pH were 4.5, 4.1, 4.7, and 0.3 g/g protein, respectively (data not shown). There was no significant difference among the RPCs, but the egg white WHC was significantly lower than those of the RPCs ($p<0.05$) (data not shown). The pH shift treatment considerably improved the WHC of RPCs through all pH values tested other than pH 6. The WHCs of the RPCs measured in

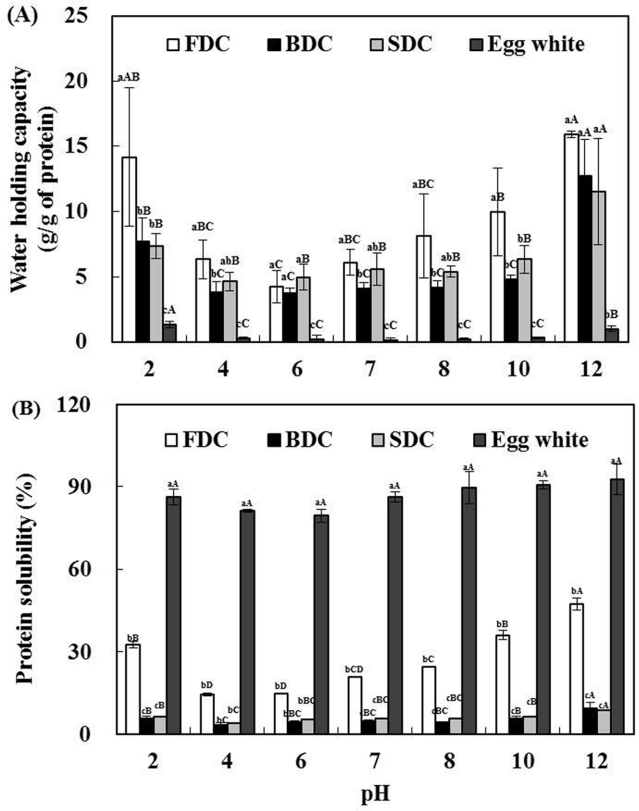

Fig. 2. Water holding capacity (A) and protein solubility (B) of roe protein concentrates (RPCs) prepared from yellowfin tuna by cook-dried process at various pH (2-12). Data represent the mean±SD of n=3. Values with different letters within the sample (small letter) and pH (capital letter) are significantly different at p<0.05 by Duncan's multiple range test.

this study were higher than those of *Labeo rohita* fish egg protein (27) and mrigal egg protein (19) concentrates. Galla *et al*. (4) reported that fish roe protein concentrate absorbs more water than oil, a property that may be attributable to the presence of polar groups in the protein concentrate that binds water molecules. However, at pH 6, protein aggregation and precipitation were enhanced, leading to a significant (p<0.05) drop in the WHC. Tan *et al*. (28) postulated that the absence of polar amino acid groups on the surfaces of the protein molecules may explain this phenomenon; these polar groups are responsible for protein–water interactions.

Protein solubility Solubility is an important functional property of proteins because it can affect other functional properties, such as rheology, hydrodynamics, and surface-active characteristics. Good solubility is crucial for many protein-based formulations (29). The solubility of the RPCs and egg white during pH shifts ranging from 2 to 12 and under conditions of stable pH are depicted in Fig. 2B. The protein solubility of the FDC (14.2%) without pH shift was higher than that of the BDC (5.4%) and SDC (5.5%). These values were significantly lower than that (80.3%) of egg white, which was used as the positive control (p<0.05) (data not shown).

Table 1. Foaming capacity (%) of yellowfin tuna roe concentrates (FDC, BDC, and SDC) prepared by cook-dried process

Sample	FDC	BDC	SDC	Egg white
Control	156.8±8.1$^{aA1)}$	109.7±2.4^{cA}	109.4±1.6^{cABC}	126.6±13.1^{bAB}
pH 2	152.1±38.8^{aA}	115.9±6.0^{aA}	112.3±6.2^{aA}	136.5±5.0^{aAB}
pH 4	137.1±27.3^{aA}	107.7±3.8^{bA}	104.5±0.9^{bC}	131.0±6.5^{abB}
pH 6	154.4±24.1^{aA}	109.5±3.6^{bA}	105.8±0.3^{bBC}	131.6±16.9^{abB}
pH 7	147.1±34.9^{aA}	108.1±1.7^{aA}	109.8±2.0^{aC}	125.1±17.0^{aAB}
pH 8	146.6±28.8^{aA}	111.1±1.7^{bA}	108.0±1.8^{bABC}	129.4±17.0^{abAB}
pH 10	162.7±37.7^{aA}	109.4±2.1^{bA}	108.6±2.1^{bBC}	133.4±18.9^{abAB}
pH 12	161.1±38.1^{aA}	114.8±3.4^{bA}	112.2±3.6^{bAB}	172.3±28.1^{aAB}

[1)]Values represent the mean±SD of n=3. Means with different small letters within same row and capital letters within same column are significantly different at p<0.05 by Duncan's multiple range test.

Close to the isoelectric point of pH 4, the FDC had the highest solubility among the RPCs (p<0.05), likely because the limited solubilization of protein promoted the release of low-molecular-weight peptides and improved the flexibility of the protein. The pH-dependent solubility of proteins is important for associated functional properties and applications in food systems (30), especially at pH values <4 or >7. The BDC and SDC had low solubility across the pH range. The proteins probably unfolded and dissociated because of the effects of acid- and alkali-limited protein solubilization, which exposed more hydrophobic residues (29). The FDC had the highest solubility, reaching 47.5% at pH 12; the high solubility probably resulted from the disassembly of tight protein structures and insoluble aggregates, which exposed many charged and polar groups to the surrounding water (31). The solubilities of the BDC and SDC reached 9.5 and 8.6%, respectively, at pH 12; these values were significantly lower than that of FDC (p<0.05), probably because the cooking processes used to obtain the BDC and SDC samples exposed the hydrophobic domains and caused heat denaturation, which reduced protein solubility (32).

Foaming capacity (FC) and foam stability (FS) Among food functionalities, FC and FS provide unique properties, such as a feeling of refreshment, food softening and dispersal of the aroma constituent. RPC FCs and FSs of 1% (w/v) are listed in Table 1 and depicted in Fig. 3. The FCs of the FDC, BDC, and SDC at the initial pH in distilled water were 156.8, 109.7, and 109.4%, respectively. The FDC FC (126.6%) was significantly higher than that of egg white (positive control; p<0.05). The FDC at the initial pH had high FS, even after whipping for 15 min (79.4%), 30 min (64.9%), and 60 min (58.8%). However, the BDC and SDC had no FS; the foam layers of these samples disappeared after whipping, perhaps as a result of elevated protein aggregation caused by heat treatment, which was indicated by the reduced solubilities (Fig. 2B) (19). Egg white had high FS, even after whipping for 15 min (93.4%), 30 min (88.5%), and 60 min (84.9%). The FCs of the FDCs across the pH values tested were in the range 131.7–162.7%. The FCs of the RPCs were not significantly different across the pH range. The FDC also had good FS,

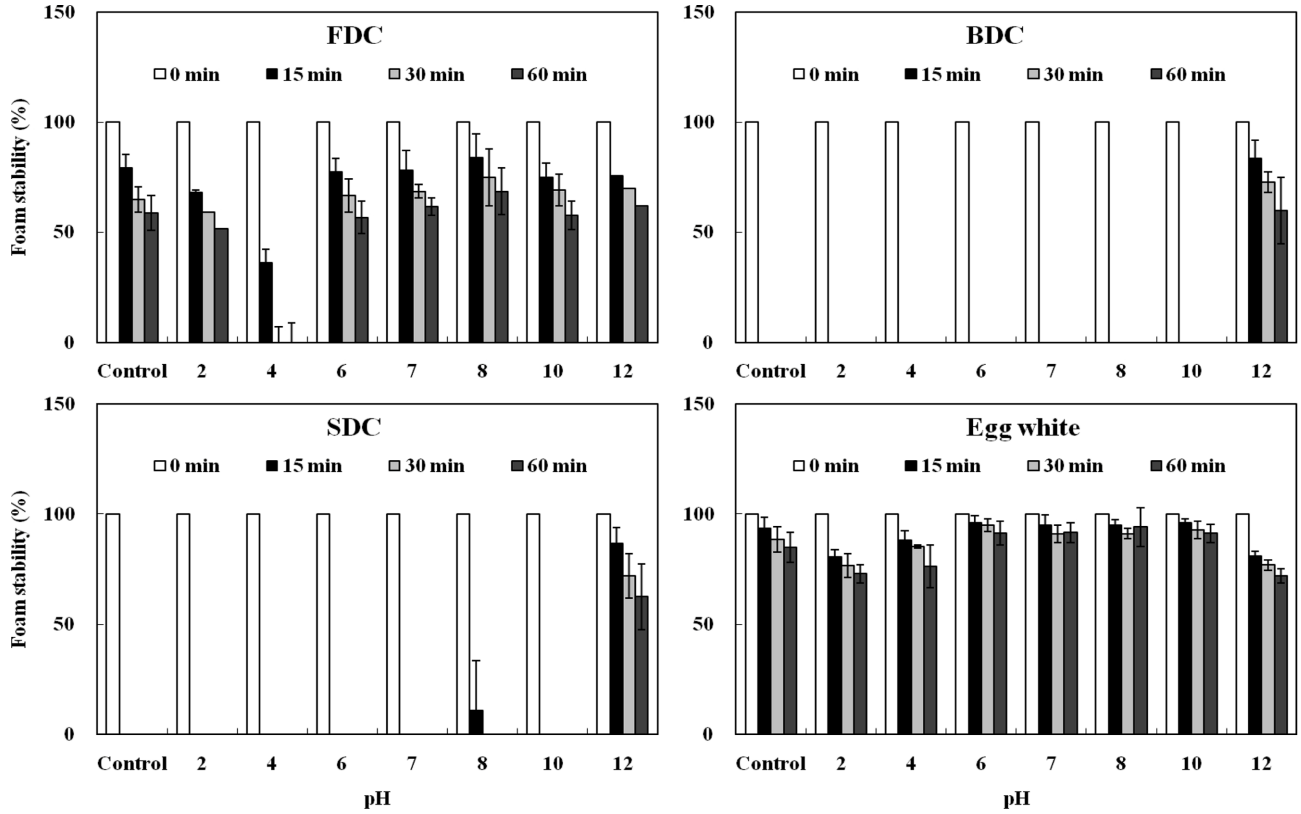

Fig. 3. Foam stability of roe protein concentrates (FDC, BDC, and SDC) prepared from yellowfin tuna by cook-dried process at various pH (2-12). Data represent the mean±SD of *n*=3.

except at pH 4 (probably a result of the elevated protein aggregation near the isoelectric point; Fig. 2B). The FS values of the BDC and SDC were lower than that of the FDC in the pH range 2–12. The FC and FS of egg white were significantly higher than those of the RPCs, especially at pH 12 (172.3%; $p<0.05$). Thus, the FC and FS values of the RPCs were affected by protein solubility. The most efficient way of increasing FC and FS was to avoid cooking the concentrates. In comparison with proteins that adsorb slowly and resist unfolding during bubbling at the newly created air/liquid interface, enhanced foaming is obtained from proteins that adsorb rapidly at the interface and undergo unfolding and molecular rearrangement (33).

Oil-in-water emulsifying activity index (EAI) and emulsion stability index (ESI) EAI (m^2/ protein) and ESI (min) values of the RPCs (1% water suspension) in the pH range 2–12 are listed in Table 2 and depicted in Fig. 4. The EAI (12.2 m^2/g protein) of the FDC at the initial pH of the sample (control) in distilled water was higher than those of the BDC and SDC (2.5 and 2.3 m^2/g protein, respectively). However, the EAI of egg white (14.7 m^2/g protein) was significantly higher than those of the RPCs ($p<0.05$). The ESI of the FDC (15.7 min) at the initial pH was lower than that of egg white (19.7 min). The EAIs of the RPCs were the highest at pH 12 ($p<0.05$). At this pH, the FDC had the highest EAI (37.2 m^2/g protein), followed in rank order by the BDC and SDC (21.9 and 15.4 m^2/g protein, respectively). Thus, the concentrate that had not been denatured had higher EAI than the cook-dried concentrates had. However, the EAIs of the RPCs were lower than that of egg white at pH 2. High EAI values of the RPCs are associated with high solubilities (34). RPCs with high protein solubility are able to rapidly diffuse and adsorb at the oil/water interface.

Table 2. Emulsifying activity index (EAI, m^2/g of protein) of yellowfin tuna roe concentrates (FDC, BDC, and SDC) prepared by cook-dried process

Sample	FDC	BDC	SDC	Egg white
Control	$12.2\pm2.1^{bCD1)}$	2.5 ± 0.4^{cBC}	2.3 ± 0.5^{cD}	14.7 ± 0.7^{aABC}
pH 2	15.6 ± 1.5^{aAC}	5.2 ± 3.1^{bC}	5.9 ± 1.7^{bBC}	18.0 ± 2.6^{aAB}
pH 4	10.7 ± 2.0^{bD}	1.2 ± 0.7^{cC}	1.5 ± 0.4^{cD}	16.2 ± 1.2^{aABC}
pH 6	14.2 ± 2.0^{bCD}	2.4 ± 0.3^{cC}	3.5 ± 0.7^{cCD}	16.9 ± 1.4^{aABC}
pH 7	10.8 ± 1.5^{bD}	2.7 ± 0.3^{cC}	3.6 ± 0.8^{cCD}	16.3 ± 1.6^{aABC}
pH 8	12.6 ± 1.7^{bCD}	3.0 ± 0.3^{cC}	4.8 ± 1.1^{cBCD}	16.6 ± 1.9^{aABC}
pH 10	21.6 ± 1.7^{aAB}	8.6 ± 1.9^{cB}	7.0 ± 1.3^{cB}	14.2 ± 1.9^{bC}
pH 12	37.2 ± 2.8^{aA}	21.9 ± 4.4^{bA}	15.4 ± 4.1^{cA}	26.2 ± 2.0^{bA}

[1)]Values represent the mean±SD of *n*=3. Means with different small letters within the same column and capital letters within same row are significantly different at $p<0.05$ by Duncan's multiple range test.

The lowest EAIs for the FDC, BDC, and SDC (10.7, 1.2, and 1.5 m^2/g protein, respectively) were measured at pH 4. At pH 12, the ESI values of the FDC, BDC, and SDC were 25.0, 18.2, and 20.3 min, respectively. Mutilangi *et al*. (34) reported that elevated contents of high-molecular-weight peptides or strongly hydrophobic peptides

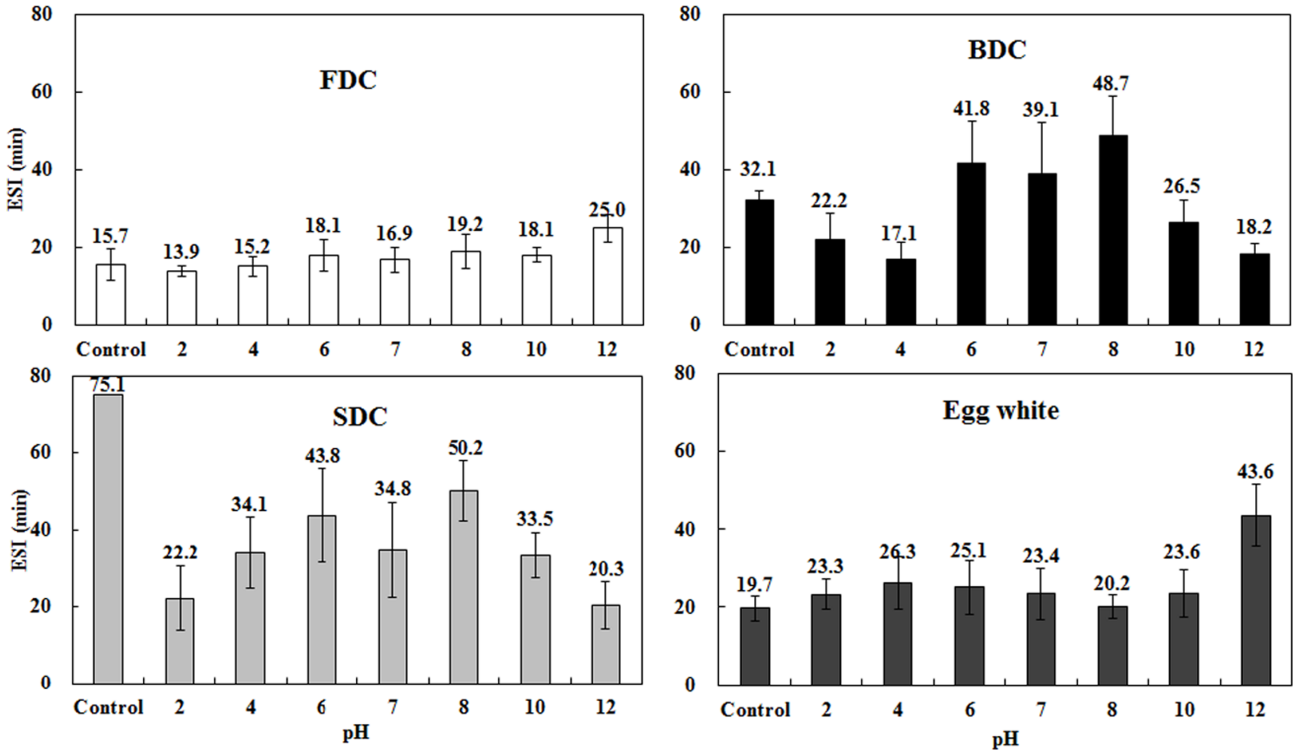

Fig. 4. Emulsion stability index (ESI) of RPCs from yellowfin tuna prepared by cook-dried process with various pH (2-12). Data represent the mean±SD of *n*=3.

contribute to the stability of the emulsions. Peptides with low molecular weights may not be sufficiently amphiphilic for good emulsification (35).

Production of concentrates from yellowfin tuna roe is a simple and economical process. The roe protein concentrates were found to be rich in protein with essential amino acids and suitable functional characteristics for supplementing in bakery and traditional foods. Therefore, these protein concentrates could be used as protein supplements and functional ingredients in human diets. This study also identifies opportunities for development of value-added products from the yellowfin tuna roe, which is currently underutilized in the fish industry.

Production of concentrates from yellowfin tuna roe by cook-dried method is a simple and economical process. The roe protein concentrates were found to be rich in protein with essential amino acids and suitable functional characteristics for supplementing in surimi based products and traditional foods. Therefore, these protein concentrates could be used as protein supplements and functional ingredients in human diets. This study also identifies opportunities for development of value-added products from the yellowfin tuna roe, which is currently underutilized in the fish industry.

Acknowledgments This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2014R1A1A4 A01008620).

Disclosure The authors declare no conflict of interest.

References

1. Ministry of Ocean and Fisheries. Yearbook of marine resource. Available from: http://www.mof.go.kr/article/view.do?articleKey=5197&boardKey=32&menuKey=396&currentPageNo=1. Accessed Jan. 3, 2015.
2. Benjakul S, Morrissey M. Protein hydrolysates from Pacific whiting solid wastes. J. Agr. Food Chem. 45: 3423-3430 (1997)
3. Galla NR, Pamidighantam PR, Akula S, Karakala B. Functional properties and *in vitro* antioxidant activity of roe protein hydrolysates of *Channa striatus* and *Labeo rohita*. Food Chem. 135: 1479-1484 (2012)
4. Galla NR, Karakala B, Akula S, Pamidighantam PR. Physico-chemical, amino acid composition, functional and antioxidant properties of roe protein concentrates obtained from *Channa striatus* and *Lates calcarifer*. Food Chem. 132: 1171-1176 (2012)
5. Kim SK, Wijesekara I. Development and biological activities of marine-derived bioactive peptides. J. Funct Foods 2: 1-9 (2010)
6. Manni L, Ghorbel-Bellaaj O, Jellouli K, Younes I, Nasri M. Extraction and characterization of chitin, chitosan, and protein hydrolysates prepared from shrimp waste by treatment with crude protease from *Bacillus cereus* SV1. Appl. Biochem. Biotech. 162: 345-357 (2010)
7. Ngo DN, Lee SH, Kim MM, Kim SK. Production of chitin oligosaccharides with different molecular weights and their antioxidant effect in RAW 264.7 cells. J. Funct. Foods 1: 188-198 (2009)
8. Kaitaranta JK, Lamppu R, Linko RR. Amino acid content of baltic herring and rainbow trout roe. J. Agr. Food Chem. 28: 908-911 (1980)
9. Sikorski ZE. The contents of proteins and other nitrogenous compounds in marine animals. In: Seafood Proteins. Sikorski ZE, Pan BS, Shahidi F (eds). Chapman and Hall, New York, NY, USA. pp. 6-12 (1994)
10. Cordero-de-los-Santos MY, Osuna-Castro JA, Borodanenko A, Paredes-López O. Physicochemical and functional characterisation of amaranth (*Amaranthus hypochondriacus*) protein isolates obtained by isoelectric precipitation and micellisation. Food Sci. Technol. Int. 11: 269-280 (2005)
11. Pires C, Costa S, Batista AP, Nunes MC, Raymundo A, Batista I. Properties of protein powder prepared from Cape hake by-products. J. Food Eng. 108: 268-

275 (2012)
12. Bellagha S, Amami E, Farhat A, Kechaou N. Drying kinetics and characteristic drying curve of lightly salted sardine (*Sardinella aurita*). Dry. Technol. 20: 1527-1538 (2002)
13. Bala BK, Mondol MRA. Experimental investigation on solar drying of fish using solar tunnel dryer. Dry. Technol. 19: 427-436 (2001)
14. Duan ZH, Zhang M, Tang J. Thin layer hot-air drying of bighead carp. Fisheries. Sci. 23: 29-32 (2004)
15. Adebiyi AP, Adebiyi AO, Hasegawa Y, Ogawa T, Muramoto K. Isolation and characterization of protein fractions from deoiled rice bran. Eur. Food Res. Technol. 228: 391–401 (2009)
16. MacDonald G, Lanier TC, Carvajal PA. Stabilization of proteins in surimi. pp. 91-125. In: Surimi and Surimi Seafood. Park JW (ed). Marcel Dekker Inc., New York, NY, USA (2000)
17. Sathivel S, Bechtel PJ, Babbitt JK, Prinyawiwatkul W, Negulescu II, Reppond KD. Properties of protein powders from arrowtooth flounder (*Atheresthes stomias*) and herring (*Clupea harengus*) byproducts. J. Agr. Food Chem. 52: 5040-5046 (2004)
18. Sathivel S, Bechtel PJ. Properties of soluble protein powders from Alaska pollack (*Theragra chalcogramma*). Int. J. Food Sci. Tech. 41: 520-529 (2006)
19. Chalamaiah M, Balaswamy K, Narsing Rao G, Prabhakara Rao PG, Jyothirmayi T. Chemical composition and functional properties of mrigal (*Cirrhinus mrigala*) egg protein concentrates and their application in pasta. J. Food Sci. Tech.-Mys 50: 514-520 (2013)
20. Lee HJ, Park SH, Yoon IS, Lee GW, Kim JS, Heu MS. Chemical composition of protein concentrate prepared from yellowfin tuna *Thunnus albacores* roe by cook-dried process. Korean J. Fish. Aquat. Sci. 19: 35-42 (2016)
21. Galla NR, Dubasi GR. Chemical and functional characterization of gum karaya (*Sterculia urens* L.) seed meal. Food Hydrocolloid. 24: 479-485 (2010)
22. Shahidi F, Han XQ, Synowiecki J. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chem. 53: 285–293 (1995)
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275 (1951)
24. Thiansilakul Y, Benjakul S, Shahidi F. Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). Food Chem. 103: 1385-1394 (2007)
25. Pearce KN, Kinsella JE. Emulsifying properties of proteins. Evaluation of a turbidimetric technique. J. Agr. Food Chem. 26: 716–723 (1978)
26. Mohamed BKF, Xia W, Issoufou A, Qixing J. Influence of pH shift on functional propertiesof protein isolated of tilapia (*Oreochromis niloticus*) muscles and of soy protein isolate. Food Bioprocess Tech. 5: 2192-2200 (2012)
27. Balaswamy K, Jyothirmayi T, Rao DG. Chemical composition and some functional properties of fish egg (roes) protein concentrate of rohu (*Labeo rohita*). J. Food Sci. Tech.-Mys 44: 293–296 (2007)
28. Tan ES, Ngoh YY, Gan CY. A comparative study of physicochemical characteristics and functionalities of pinto bean protein isolate (PBPI) against the soybean protein isolate (SPI) after the extraction optimisation. Food Chem. 152: 447-455 (2014)
29. Yuan B, Ren JY, Zhao MM, Luo DH, Gu LJ. Effects of limited enzymatic hydrolysis with pepsin and high-pressure homogenization on the functional properties of soybean protein isolate. LWT-Food Sci. Technol. 46: 453-459 (2012)
30. Kinsella JE. Functional properties of soy proteins. J. Am. Oil Chem. Soc. 56: 242-258 (1979)
31. Kristinsson HG, Rasco BA. Biochemical and functional properties of Atlantic salmon (*Salmo salar*) muscle hydrolyzed with various alkaline proteases. J. Agr. Food Chem. 48: 657-666 (2000)
32. Sikorski ZE, Naczk M. Modification of technological properties of fish protein concentrates. Crit. Rev. Food Sci. 14: 201-230 (1981)
33. Damodaran S. Protein-stabilised foams and emulsions. pp. 57-110. In: Food Proteins and Their Applications. Damodaran S, Paraf A (eds). Marcel Dekker, New York, NY, USA (1997)
34. Mutilangi WAM, Panyam D, Kilara A. Functional properties of hydrolysates from proteolysis of heat-denatured whey protein isolate. J. Food Sci. 61: 270-275 (1996)
35. Chobert JM, Bertrand-Harb C, Nicolus MG. Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. J. Agr. Food Chem. 36: 883-892 (1988)