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Functional Hydrolysates from Yellow Fin Tuna Red Meat Using RSM Based Optimization

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The major hydrolytic variables for deriving functional hydrolysates from red meat of yellow fin tuna (*Thunnus albacares*) were optimized using response surface methodology (RSM). A central composite design (CCD) was employed to obtain the best possible combination of enzyme-substrate ratio (X_1: 0.25-1.5%) and hydrolysis duration (X_2: 30-240 min) to derive hydrolysates with strongest functional properties with emphasis on protein recovery and sensory acceptability. The experimental data obtained were fitted to second-order and third-order polynomial equation using multiple regression analysis. The optimum hydrolytic conditions for superior functional properties were achieved at an enzyme-substrate ratio (E/S) of 0.34 % for a hydrolysis duration of 30 minutes. The protein recovery of the hydrolysates ranged between 32 - 50% which were linearly related ($R^2 = 0.67$) to the degree of hydrolysis. However a few properties of hydrolysate having the same degree of hydrolysis varied significantly and hence, could not be entirely explained based on the degree of hydrolysis.

Introduction

Globally, tuna resources have high commercial value on account of its demand for thermally processed delicacies. However, the process discards generated during canning operations including the red meat is enormous and accounts to about 50 to 70% of whole fish (Saidi *et al.,* 2014). Reports by Sutanbawa and Aknes (2006), revealed an estimate of 4,50,000 million tons per year of processing discards globally from the tuna canning industry. Recovery and conversion of these proteinaceous discards to functional and bioactive hydrolysates is a promisive option for the industry. These hydrolysates containing peptides with distinct range of molecular weight, exhibit superior functional and bioactive properties compared to that of parent protein (He *et al.,* 2013; Binsi *et al.,* 2016). Based on the extent of hydrolysis that the parent protein undergoes, the properties exhibited by the hydrolysates vary considerably.

The attempts for arriving at an optimum degree of hydrolysis (DH) using response surface methodology (RSM) have been made

extensively on various substrates considering nitrogen recovery (Awuor *et al.,* 2017), bioactive (Wangtueai *et al.,* 2016; Wang *et al.,* 2017) and functional properties (Jamil *et al.,* 2016), as process responses. However, it is well understood that the properties of hydrolysates depend to a large extent on the nature of polypeptide fragments formed, rather than the DH achieved during the hydrolytic process. It is quite obvious that peptides from the same source having the same DH exhibit significant variations in their properties.

At present, the red meat generated during tuna canning operations is mainly converted to low value by-products like animal feed, fertilizers etc.

Hence, the current investigation was intended towards optimizing the effect of hydrolysis variables viz., E/S and hydrolysis duration, using an RSM based central composite design, for extraction of functional hydrolysates from the cannery waste; cooked meat of yellow fin tuna red meat with thrust to maximum protein recovery.

Moreover, a variability range of DH values with respect to individual properties exhibited by hydrolysates under each hydrolytic conditions was derived by statistical means.

Materials and Methods

Raw material and chemicals

Tuna red meat was collected as by-product after canning and retorting from Forstar Frozen Foods Pvt. Ltd., Taloja, Navi Mumbai, which had undergone prior heat treatment at 121 ^{o}C for 1 hr. It was initially washed with boiled water (1:4 (w/v)) for five minutes, pressed and further subjected to washing with cold 0.2% (w/v) sodium bicarbonate solution (1:4 (w/v)) for two minutes and pressed to remove excess moisture. This washed meat

was used as the starting material for the preparation of protein hydrolysates. Papain enzyme (Hi Media, India) from papaya latex was used for hydrolysis. All other chemicals used for the study were of analytical grade.

Preparation of protein hydrolysate

The washed red meat was comminuted thoroughly using an electric blender, weighed and added with twice the amount of water for each run. A preliminary trial was carried out for optimizing the temperature of hydrolysis considering degree of hydrolysis, protein recovery and proteolytic activity as process variables with major parameters viz. pH, enzyme: substrate ratio (E/S) and hydrolysis time as constant (Data not shown).

Further experiments based on the statistical design were performed in a shaking water bath (Shaking bath, Neolab Instruments, Mumbai, India) with continuous agitation at this constant optimised temperature. On completion of the process, the hydrolysis was terminated by heating the solution to 85-90 °C for 15-20 min. The resultant solution was cooled and centrifuged at 8000 g at 10°C for 20 min (K-24A, Remi Instruments, Mumbai) to obtain supernatant which was further used for analysis.

Experimental design

Response Surface Methodology with a central composite design (CCD) with two independent variables at three levels was chosen based on the results of preliminary experiments. The input factors were enzyme-substrate ratio (E/S) (X_1) and hydrolysis time (X_2). pH was maintained constant and optimized hydrolysis temperature was adopted. Single and combined effects of the variables on the responses were studied by formulating thirteen experimental runs. The responses included protein recovery, foaming,

emulsifying, oil absorption and sensory properties for functionality optimization. Contour plots and response surface graphs were generated by the predictive model to envisage the critical points and the effectiveness of each factor. Desirability score was computed for multi response optimization of response variables for functionality of hydrolysate with emphasis to protein recovery and the optimum combination of enzyme substrate ratio and hydrolysis time was selected.

Determination of proximate composition

Proximate composition of tuna red meat before and after treatment was estimated as per AOAC (2012). Protein content of tuna red meat and hydrolysates were estimated by kjelhdahl method.

Determination of Degree of Hydrolysis (DH)

Degree of hydrolysis was estimated as per the methodology described by Hoyle and Merritt (1994). Briefly, degree of hydrolysis was computed as

$$\% \ DH = \frac{10\% \ TCA \ soluble \ N_2 \ in \ the \ sample}{Total \ N_2 \ in \ the \ sample} \times 100$$

Determination of protein recovery

Protein recovery in hydrolysate was defined as the percentage of protein obtained during the extraction process to the total amount of protein in raw material and was calculated as follows:

$$Recovered \ protein \ (\%) = \frac{Protein \ in \ hydrolysate \times Volume \ of \ hydrolysate}{Weight \ of \ red \ meat \ taken \times Amount \ of \ protein \ in \ red \ meat} \times 100$$

Determination of functional properties

Foaming properties

Foaming capacity and stability of fish protein hydrolysate were determined by the method described by Sathe and Salunkhe (1981). Protein solution (1.0 %) was homogenized (230 VAC T-25 digital Ultra-turrax, IKA, India) at a speed of 16,000 rpm for 2 min to entrap air and foaming capacity was determined instantly whereas foam stability after a time period of 3 minutes as:

$$Foaming \ capacity/stability \ (\%) = [(A-B) \ / \ B] \times 100$$

Where A is the volume immediately after whipping (foam capacity) and after 3 min standing (foam stability); B is the volume before whipping.

Emulsifying properties

Emulsifying properties were determined according to the method of Pearce and Kinsella (1978). A pre-mix containing 1% protein solution and vegetable oil (3:1 (v/v)) were homogenized (230 VAC T-25 digital Ultra-turrax, IKA, India) for a period of 1 min at 20,000 rpm and an aliquot of the emulsion (50 μl) was carefully taken from the bottom of the container at 0 and 10 min after homogenization. Further it was mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution and the absorbance measured at 500 nm (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore) immediately (A_0) and 10 min (A_{10}) after emulsion formation to evaluate the emulsifying activity index (EAI) and the emulsion stability index (ESI) as:

$$EAI \ (m^2/g) = \frac{2 \times 2.303 \times A_0}{0.25 \times wt \ of \ protein}$$

$$ESI \text{ (min)} = \frac{A_{10} \times \Delta t}{\Delta A}$$

Where $\Delta A = A_0 - A_{10}$ and $\Delta t = 10$ min

Oil absorption capacity

OAC of sample was determined as the volume of edible oil held by a known quantity of the material as per the method of Shahidi *et al.,* (1995). A 0.5 g sample with oil added (10 ml) was vortexed (Expo Hitech, India) for 30 sec following centrifugation (K-24A, Remi Instruments, Mumbai) at 2800 g for 25 min.

The free oil was decanted and the OAC was determined by weight difference (g of oil per gram of sample).

Sensory property

Bitterness of hydrolysate was sensorily evaluated by 10 trained panellists as per Nilsang *et al.,* (2005) with modifications. The panelists were trained using caffeine solution as a standard which was presented in different reference concentrations for anchoring the bitterness scale. A 10-cm line scale anchored from ''no bitterness'' (as 1) to ''extreme bitterness'' (10) was defined.

Panellists evaluated bitter taste of hydrolysate samples in comparison with the standard reference using the designed scale.

Statistical Model Development

The CCD in the experimental design consisted of 13 experimental points conducted in random order (5 factorial points, 5 axial points and 3 center points) (Table 1). Second order/ quadratic and third order/cubic regression models were fitted to the response variables as a function of input variables using the polynomial equation:

Second order regression: $Y = \beta_0 + \beta_i X_i + \beta_{ij} X_i X_j + \beta_{ii} X_i^2$, $i \neq j = 1,2$

Third order regression: $Y = \beta_0 + \beta_i X_i + \beta_{ij} X_i X_j + \beta_{ii} X_i^2 + \beta_{iij} X_i^2 X_j$, $i \neq j = 1,2$

Y being the response; β_0: the offset term; β_i, β_{ij}, β_{ii} and β_{iij} being the regression coefficients and Xi and Xj, the levels of the independent variables. The performance of the fitted model was assessed by Coefficient of determination (R^2) and mean square error (MSE). Significance of the regression coefficients was determined at 5% level of significance ($p < 0.05$). A software Design expert 7.0 was used to fit the models.

Results and Discussion

Proximate composition of raw material mince

The proximate composition of tuna red meat mince before and after water washing was assessed. Tuna red meat was initially washed with boiled water and subsequently with 0.2% cold sodium bicarbonate solution to remove excess of fat and pigments. Previously, Bhaskar *et al.,* (2008) suggested a prior hot water washing (85° C for 20 min) for catla visceral waste followed by centrifugation to increase the stability of the hydrolysates towards lipid oxidation.

The washing process increased the moisture content of mince by 3.38 % (67.11 ± 0.02 to 70.49 ± 0.48 %), with a proportional decrease in the protein content from 28.19 ± 0.62 to 24.98 ± 0.24 %. This was also accompanied by a significant ($p < 0.05$) reduction in the fat (2.22 ± 0.02 % to 1.4 ± 0.17 %) and ash content (1.43 ± 0.06 to 0.64 ± 0.01 %) of the mince to almost half of its initial value. The higher moisture content observed in the washed mince might be either due to the hydration of myofibrillar proteins or may be a

relative increase associated with the loss of water soluble proteins, fat and mineral during the leaching process.

Optimization of hydrolysis parameters

The optimisation of hydrolytic parameters was carried out in two steps. Initially, the temperature for the hydrolysis was optimised keeping E/S, pH and time as constant, followed by the optimisation of E/S (X_1) and hydrolysis time (X_2) under optimum conditions of temperature and pH. The response variables considered for optimising the process temperature were degree of hydrolysis, protein recovery and proteolytic activity. All the variables were found to be increasing upto 60°C, and thereafter showed a decreasing trend. Hence, the subsequent hydrolysis experiment was carried out at a single temperature of 60°C. Similarly, the pH opted for the hydrolysis process was the initial pH of the substrate (washed mince), ie pH 6.5, since it falls within the optimal pH range of papain enzyme as indicated by the manufacturer (pH 6-7).

RSM based optimisation of process variables

The influence of E/S (X_1) and hydrolysis time (X_2) under optimum conditions of temperature and pH by papain on cooked tuna red meat protein was determined using central composite design. The response variables considered for optimising the derivation of functional hydrolysate were foaming, emulsifying and oil absorption properties together with sensory property. For optimization, protein recovery was also given special emphasis. A multiple regression analysis technique was performed to determine all the coefficients of linear (X_1, X_2), quadratic (X_1^2, X_2^2) and interaction (X_1X_2) terms to fit a full response surface model for the responses.

Changes in DH

Previously, several authors have suggested degree of hydrolysis as the major contributor to the specific properties exhibited by the peptides (Ren *et al.,* 2008; Taheri *et al.,* 2013). However, peptides derived from the same source having similar DH values quite often vary in their properties. Authors like Amarowicz (2008) have suggested that the presence of specific peptides liberated from protein as well as the amount of free amino acids to be important in determination of bioactive properties of protein hydrolysate. Hence, in the present study, DH was not included in the RSM analysis matrix. However the DH values were independently determined for each hydrolytic conditions mentioned in the matrix. Further, the changes in the individual properties were discussed in relation to the changes in DH values.

Variations in protein recovery

The protein recovery was considered as a response variable for both the optimisation designs, as it adds to the economics of the hydrolysis operation. The variations in this response during the hydrolytic process were best explained by second order response surface model ($p < 0.05$) (Fig. 1a) with an R^2 value of 0.98 and MSE of 1.78 using the equation:

PR= 27.30 + 20.79 X_1* + 0.03 X_2 – 3.24E-003 X_1X_2 – 4.99 X_1^2 -8.52E-005 X_2^2

The adjusted R^2 of the fitted model was 0.96 and analysis of lack of fit was found to be insignificant ($p > 0.05$) indicating the suitability of the model. The precision measures of S/N (signal to noise ratio) was greater than 4 (17.797) indicating adequate model discrimination (Myers *et al.,* 2009). The linear effect of X_1 on nitrogen recovery was found to have a statistically significant

effect ($p < 0.05$) with high regression coefficient value of 20.79, whereas that of X_2 was marginal. The quadratic effects of both the variables showed negative values indicating that the protein recovery reaches a threshold level at certain value of E/S and time, thereafter showing a reduction in the rate of increase. It is generally agreed that the high E/S ratio and longer hydrolysis period favours higher protein recovery (Diniz and Martin, 1998; Liaset *et al.,* 2002). In the present study, protein recovery varied directly with the degree of hydrolysis ($R^2 = 0.67$) of up to 25% and thereafter showed a slightly decreasing or more or less similar values (Fig. 1b). However for similar DH, variations were observed in this response ranging on an average from 5 – 9 %.

It was also noticed that as indicated in quadratic equation, E/S had more influence than time with higher E/S giving more recovery of protein from substrate than the period of hydrolysis. From the regression coefficients of statically fitted models observed in the present study, it may be inferred that increasing the concentration of enzyme is more beneficial in getting higher protein recovery than increasing the duration of hydrolysis beyond a DH value of 25%.

Variations in functional properties

Foaming properties

Foaming properties are usually expressed in terms of foaming capacity and foam stability. Second order regression model with an R^2 value of 0.86 explained the changes in foaming capacity of hydrolysate under different hydrolytic conditions. The regression coefficient values of fitted quadratic model for foaming properties were:

FC = $166.63 + 33.05 X_1 + 0.04 X_2* − 0.13 X_1X_2 – 8.03 X_1^2 -6.12$ E-004 X_2^2

The FC values showed strong positive linearity with X_1 (Fig. 1c), however the quadratic effect showed negative values. In the case of X_2, the linear effect on protein recovery was minimum, however was found to be statistically significant ($p < 0.05$).This essentially means that, FC values increased initially with increase in E/S ratio to reach a threshold value, and thereafter decreased with every unit of increase in E/S ratio. Foaming capacity was higher at lower DH (Fig. 1d) and it ranged from 122 - 205% under different conditions of E/S ratio and hydrolysis time which in turn influenced the DH. For similar DH it exhibited wide variations in the property ranging from 35 - 60 %, on an average.

Similar to protein recovery, E/S had higher influential role than time and hence for similar DH, the hydrolytic condition with higher E/S gave better foaming capacity for the derived hydrolysate. Variations in the foam stability were explained by quadratic regression model with an R^2 of 0.96. The foam stability values showed strong negative linear effect with X_1 while giving a positive quadratic effect, whereas both linear and quadratic effect was found to be marginal for X_2. Similarly, the interaction effects of both X_1 and X_2 on foaming properties were also minimum.

FS = $177.75 − 155.98 X_1* - 0.23 X_2* + 0.48 X_1X_2* + 26.30 X_1^2 – 1.59$E-003 X_2^2

The results suggested a drastic reduction in FS with increase in the concentration of enzyme upto certain degree of hydrolysis, thereafter showing constant values. There was a general trend of decrease in the foaming properties with increase in DH (Table 1). However, it was observed that for similar range of DH, distinctly different response were exhibited, which further suggest that the nature of peptides formed under different hydrolytic conditions play a major role in determining the final properties of hydrolysate.

Table.1 Experimental design and responses of the dependent variables to the hydrolysis conditions

Design point[a]	X_1	X_2	DH	PR	FC	FS	EAI	ESI	OAC	Bitterness
1	0.25	30	12.42	32.66	160	130	182.4	37.01	1.23	3
2	0.88	240	30.78	43.44	130	25	75.95	23.41	1.32	9
3	1.5	240	38.10	47.13	130	30	70.54	25.36	1.25	10
4	1.5	30	24.65	49.89	180	25	97.34	33.92	1.38	8
5	1.5	135	31.18	48.45	162	30	74.01	28.48	1.34	9
6	0.25	135	18.54	35.26	167	105	107.39	20.33	1.23	5
7	0.25	30	12.43	32.90	177	145	176.99	33.98	1.27	4
8	1.5	30	24.66	46.24	198	20	90.74	29.15	1.35	8
9	1.5	240	39.12	50.11	130	20	67.43	28.84	1.28	10
10	0.88	30	20.98	42.63	205	48	92.98	32.65	1.22	7
11	0.25	240	20.17	33.95	154	20	101.73	34.43	1.27	6.5
12	0.88	240	30.92	43.14	122	20	75.95	23.42	1.37	9.5
13	0.25	240	20.58	34.42	133	10	108.24	31.68	1.23	6

[a]Experiments were run at random, X_1: Enzyme-substrate ratio (%), X_2: Hydrolysis time, DH: Degree of hydrolysis (%), PR: Protein recovery (%), FC: Foaming Capacity (%), FS: Foam Stability (%), EAI: Emulsifying Activity Index (m^2/g), ESI: Emulsion Stability Index (min), OAC: Oil Absorption Capacity (g/g)

Fig.1 Variations in protein recovery (%) a. in response to enzyme-substrate ratio and hydrolysis time; b. in relation to DH; Variations in Foaming capacity (%) c. in response to enzyme-substrate ratio and hydrolysis time; d. in relation to DH; Variations in Emulsifying Activity Index (m^2/g) e. in response to enzyme-substrate ratio and hydrolysis time; f. in relation to DH; Variations in Oil Absorption Capacity (g/g) g. in response to enzyme-substrate ratio and hydrolysis time; h. in relation to DH; Variations in Bitterness i. in response to enzyme-substrate ratio and hydrolysis time; j. in relation to DH

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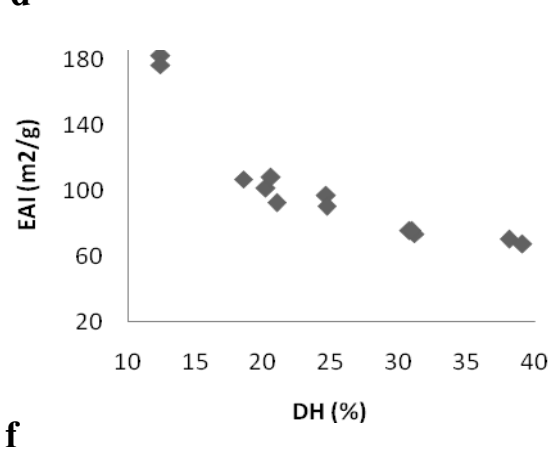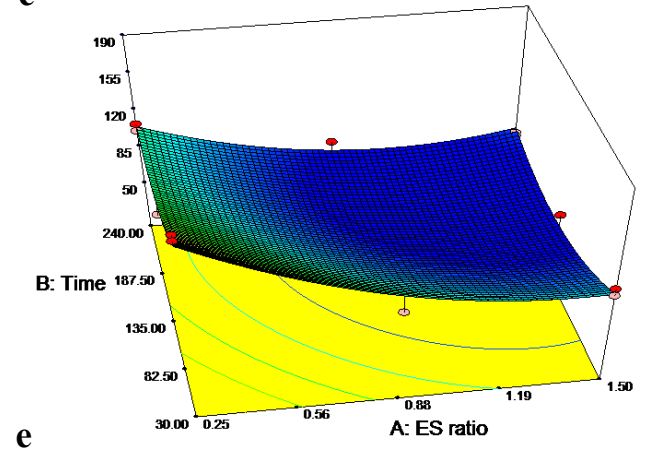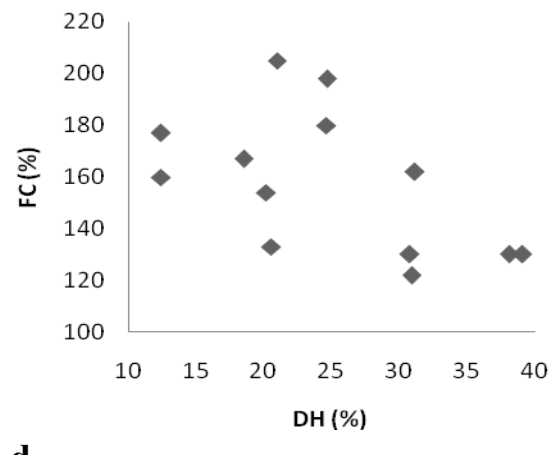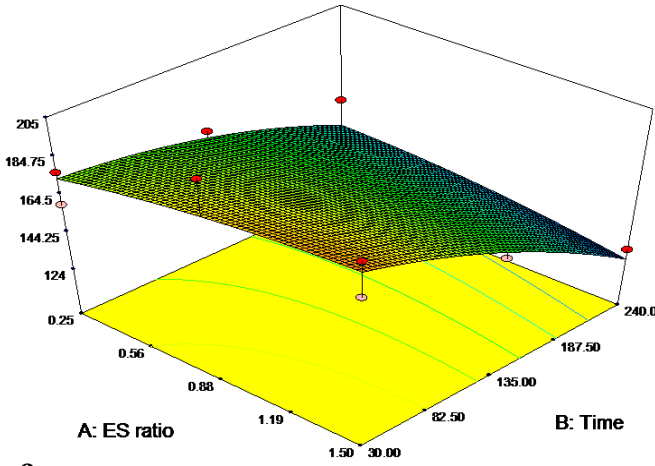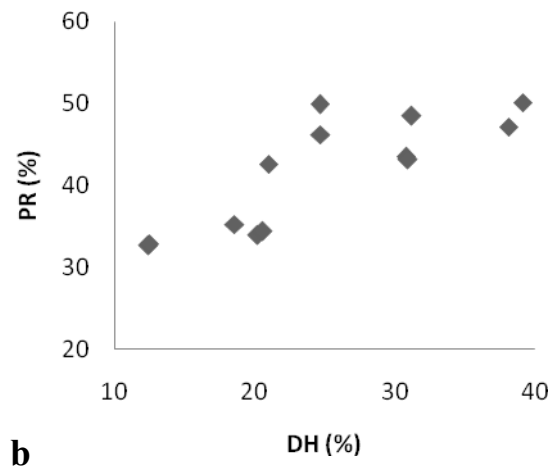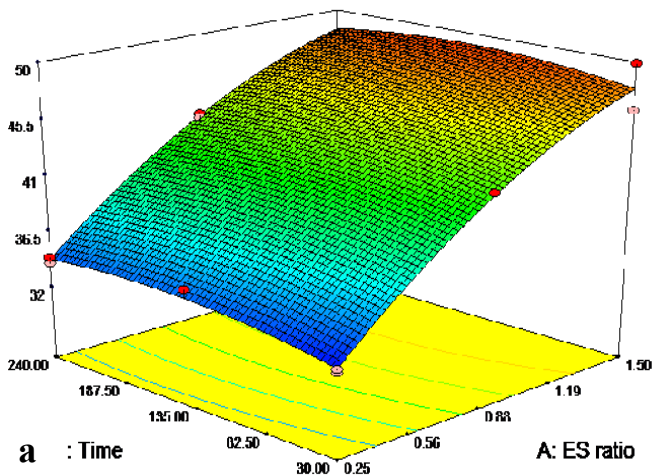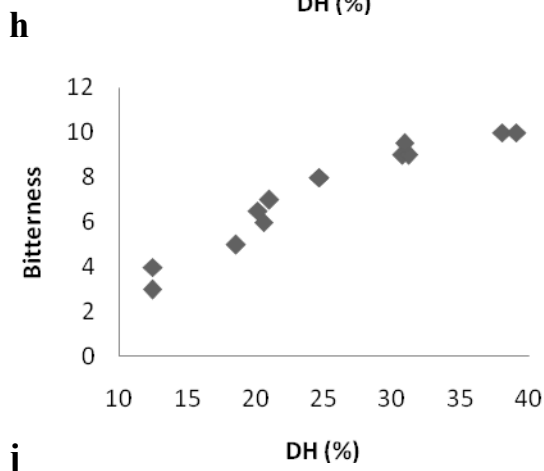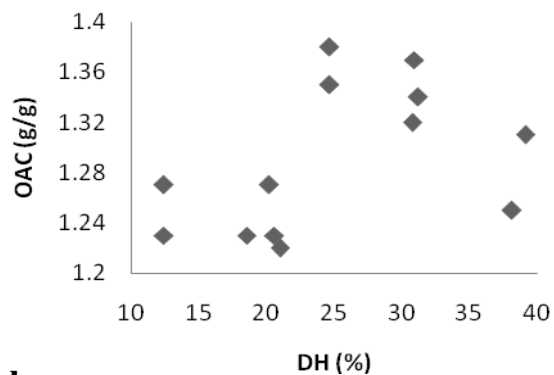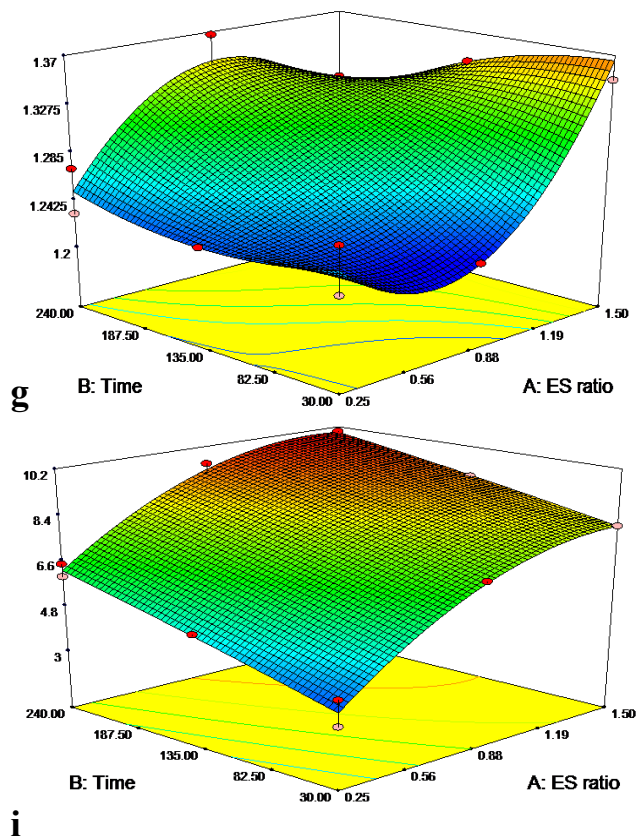
Emulsifying properties

Second order regression model was best fitted for explaining the changes in EAI. The high coefficient of determination (R^2 = 0.95) indicated that the model as fitted can explain 94.6% of the EAI variability (Fig. 1e). Linear, second order and interaction of both X_1 and X_2 were found to be significant (p < 0.05) in influencing the variations of this response.

EAI = −235.98 − 173.63 X_1* - 0.88 X_2* + 0.19 X_1X_2* + 59.29 X_1^2* + 1.93 E-003 X_2^2

Both the factors were inversely related to the changes in response as indicated by negative regression coefficient. However linear as well as quadratic terms of X_1 was observed to be more important for response variations indicating a regression coefficient of 173.63 and 59.29, respectively while X_2 showed only a marginal influence on EAI.

ESI was explained using cubic model with an R^2 of 0.92. An adequate precision of 8.510, indicating the signal to noise ratio was observed and as the value was greater than the desired value of 4, the present model indicated its fitness for explaining the variations in ESI. The linear terms of X_1 and X_2 as well as second order of X_2 and interaction of linear terms X_1 and second order of X_2 were the significant terms (p < 0.05). X_1 and X_2 were equally influential for the variations in this response however, X_1 was directly related while X_2 was inversely related to ESI in hydrolysate.

The equation explaining the variations in ESI in terms of coded factors was:

ESI = 20.66 + 4.08 X_1* − 4.62 X_2* − 0.50 X_1X_2 + 3.75 X_1^2 + 7.39 X_2^2* + 2.90 $X_1^2 X_2$ − 6.55 $X_1 X_2^2$*

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Both EAI and ESI indicated significant reduction in values with progress of hydrolysis, with the highest values at a DH of 12.42% which is the lowest DH obtained during the study, corresponding to X_1 of 0.25 % and X_2 of 30 min (Fig. 1f, Table 1). In general, the emulsifying properties showed marked decrease in the values above DH value of 20%. EAI exhibited an inverse relation with DH ($R^2 = 0.75$) and variations of up to 12 m^2/g were observed for similar DH generated through different combinations of X_1 and X_2. However the variations in ESI for similar DH ranged from 0.5 min - 5 min. Kristinsson and Rasco (2000) stated to have exceptional emulsifying activity and stability for hydrolysate produced under limited degree of hydrolysis. Similar reports of greater emulsifying capacity and emulsion stability were noticeable when DH was low for salmon byproduct hydrolysate (Gbogouri *et al.,* 2004) and sardine hydrolysate (Quaglia and Orban, 1990).

Oil Absorption Capacity

OAC is related to the surface hydrophobicity in hydrolysates which is facilitated by the hydrolysis process. Cubic model with an R^2 of 0.88 best explained the variations in this response (Fig. 1g). All the influencing variables X_1 and X_2 had a marginal effect with positive correlation. However linear terms of X_2 as well as interaction effect of second order terms of E/S and linear terms of hydrolysis time ($X1^2X_2$) were significant (p < 0.05). The model was fitted using the equation in terms of coded factor as:

OAC = 1.28 + 0.056 X_1 + 0.063 X_2* − 0.023 X_1X_2 + 4.731 E–003 X_1^2 − 2.388 E-003 X_2^2 − 0.084 $X_1^2 X_2$* − 0.019 $X_1 X_2^2$

During hydrolysis, the variation in OAC was minimum ranging between 1.23 − 1.38 g/g with the changes in factors viz., X_1 or X_2

(Table 1). Unlike the foaming and emulsifying properties, higher OAC value was observed at a slightly higher DH value of 24.65%, however further decreased above DH value of 30%.The correlation studies of OAC with DH also substantiated varying response with no definite increase or decrease (Fig. 1h). Variations in the hydrophobicity of the polypeptide fragment formed during hydrolysis might have resulted in wide variations in OAC exhibited by the hydrolysate derived under similar degree of hydrolysis. Similar to this, DH ranging between 2.4 – 2.8 ml/g was reported in cobia frame hydrolysates by Amiza *et al.,* (2012) and an OAC ranging between 0.9 – 1.4 g/g was reported in hake by-product hydrolysates by Pires *et al.,* (2012).

Sensory property

Although enzymatic hydrolysis of protein develops desirable functional properties, it has the disadvantage of generating bitterness which is identified as a major hindrance in the utilization and commercialization of bioactive FPH (Kim and Wijesekara, 2010). The mechanism of bitterness is not very clear, but it is widely accepted that hydrophobic amino acids are one of the major contributors. Of the many techniques suggested to reduce or mask bitterness in hydrolysates, strict control of any hydrolysis experiment and termination at low degree of hydrolysis is desirable to prevent the development of bitter taste and retention of functional properties (Adler-Nissen, 1986; Saha and Hayashi, 2001).Quadratic regression model (p < 0.05) with a high determination coefficient ($R^2 = 0.99$) and an MSE of 0.11 was fitted to predict the trends of bitterness of hydrolysate generated with various combinations of X_1 and X_2. The adjusted R^2 value of 0.98 and predicted R^2 value of 0.96 which were in reasonable agreement, further confirms the high significance of the fitted model. Linear terms of both X_1 and X_2 as well

as second order term of X_1 were significant terms (p < 0.05) with X_1 being more influential in determining the variations of bitterness while hydrolysis time (X_2) had a marginal influence for the same extent of hydrolysis.

Bitterness = 1.07 + 8.85 X_1* + 0.02 X_2* - 2.86E-003 X_1X_2 - 2.96 X_1^2* - 5.67 E-006 X_2^2

However beyond an extended limit, X_1 had minimum influence in bitterness generation in the hydrolysates as indicated by a negative coefficient for quadratic regression terms. Response surface graphs generated by the predictive model clearly indicated the trends in the bitterness with X_1 and X_2 (Fig. 1i).

The observed trend was well in agreement with the changes in DH, as indicated by the R^2 (0.915) value of correlation graph where bitterness increased almost linearly with DH upto a value of 30% and thereafter showed a stagnating trend (Fig. 1j). The variations between the samples having same DH obtained through different combinations of X_1 and X_2 were minimum with a slightly higher influence for E/S in the generation of bitterness than period of hydrolysis which was substantiated from regression equation for this response.

From above observations, it may be inferred that optimum conditions for extracting functional peptides were; E/S of 0.34 % for a duration of 30 minutes at 60^0C and pH 6.5. The maximum desirability score obtained for this condition was 0.49, when protein recovery was included as response variable, and 0.70 without protein recovery. Further, all the response variables of the final products were validated. The experimental and predicted values were within the range and did not differ statistically at 5% level (data not shown), thus confirming the reliability of the optimised condition.

The objective of the study was to identify the optimised process conditions for deriving tuna protein hydrolysate having desirable range of functional properties by suitable statistical models. Moreover, emphasis was given to assess the dependency of these properties on degree of hydrolysis by comparing the values of hydrolysates obtained through different hydrolytic conditions, but having similar DH values. It was clear from the results that, for the parameters analysed, enzyme-substrate ratio (X_1) was more influential in explaining the response variations than hydrolysis time. Correlation studies between the degree of hydrolysis and responses recommended that though a general trend can be suggested, peptide properties can't be entirely explained based on the degree of hydrolysis but rather on the nature of polypeptide fragment formed under different hydrolytic conditions.

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