Fishery Technology 53 (2016) : 48 - 54

Empirical Modeling on the Effect of Sodium Chloride, Temperature and pH on Solubility of Myosin Extracted from *Labeo rohita*

Madhusweta Das*
Department of Agricultural and Food Engineering, Indian Institute of Technology, Kharagpur - 721 302

Abstract

The present study describes the modeling of the effect of sodium chloride concentration, temperature and pH on dissolution of myosin isolated from fresh waters fish, *Labeo rohita*. The goodness of fit was assessed by coefficient of determination, root mean square error and residual plot.The trend observed in the dissolution behavior could be well described by mathematical equations, the nature being case specific. At pH 6.2, with sodium chloride concentration as independent variable, solubility followed a four degree polynomial for temperatures ranging from 10-33°C and a three degree for 45-65°C. Maximum solubility obtainable increased linearly with decrease of temperature from 65 to 10°C, however, for the whole temperature range, dissolution attained peak at around 0.85 M sodium chloride. Solubility was also a function of pH and exhibited three degree dependency in absence of salt and a two degree dependency in presence of salt. The isoelectric pH of myosin shifted towards acidic side in presence of salt.

Keywords: Protein solubility, freshwater fish, *Labeo rohita*, myosin, empirical modeling

Introduction

Myosin is the most important muscle protein for developing adequate binding properties for desirable texture in sectioned and formed meats, emulsified and comminuted sausage products (Samejima et al., 1969; Nakayama & Sato, 1971; Macfarlane et al., 1977; Ford et al., 1978; Park, 2009; Ugalde-Benitez, 2012). Siegel & Schmidt (1979) and Saha et al (2009) opined that salt and phosphate increase binding of myosin and meat primarily by solubilizing action. Several workers have established a positive correlation between protein extractability and binding properties and its relevance to meat processing (Acton 1972; Siegel & Schmidt, 1979; Miller et al., 1980; Sen, 2005). Samejima et al (1985) and Keever (2011) have confirmed the importance of maximizing the concentration of dissolved protein in the processed meat products. Using solubility as an analytical technique, Peng et al. (1982) studied the possible protein-protein interaction between purified soybean 11 S protein and rabbit skeletal muscle myosin. Muscle protein solubility has been widely used as an analytical tool to evaluate the functional properties of ice-stored, frozen and frozen-stored fish (Geirsdottir et al., 2007; Yathavamoorthi et al., 2012). For incorporation into different products, Shaviklo (2008) used solubility as a preparative technique to isolate protein from several fishes.

The need for sodium chloride in a concentration range of 0.3-0.6 M to solubilize muscle myofibrillar protein is widely quoted (Yeong et al., 2002). However, few reports are available on solubility of muscle protein in water (Kelleher et al., 2004). Stefansson & Hultin (1994) observed that cod muscle protein is appreciably soluble in water at neutral pH at an ionic strength of 0.0003 or less. Chen & Jaczynski (2007) reported that pH for minimum solubility of rainbow trout protein gets shifted towards acidic side in presence of salts. Lin & Park (1998) and Thawornchinsombut & Park (2004) studied the solubility of salmon myosin and pacific whiting muscle proteins, respectively, as affected by conformational changes at various ionic strengths and pH. Geirsdottir et al. (2007) and Huss (1988) reported the solubility of fish protein as a

Received 18 March 2015; Revised 25 June 2015; Accepted 17 August 2015

* *E-mail: madhu@agfe.iitkgp.ernet.in*

© 2016 Society of Fisheries Technologists (India)

function of pH. In all these studies, the fish used was marine fish and no study with fresh water fish.

India stands second in the world production of fish, and 63% of total production amounts to freshwater fish (Thakur, 2014). Functionality of protein depends on source and several workers have indicated the possibility of the use of freshwater fish, carp and its derived product, in different fabricated foods (Yueh, 1975; Asghar et al. 1984; Nowsad et al.1999; Vega-Warner et al., 1999; Yapar et al., 2006; Elavarasan et al., 2014).

Das & Chattoraj (1989) studied the solubility of myosin from freshwater fish *Labeo rohita* (rohu) in presence of various salts like sodium chloride, potassium chloride and sodium sulfate. Besides the nature of salts, they reported strong dependency of solubility on the environmental condition like ionic strength, temperatures and pH values. As sodium chloride is widely used in fish processing, it would be highly desirable to have appropriate empirical model for predicting the myosin solubility for precise control over the fabricated product quality.

Though a few theoretical approaches have been reported on modeling of solubility of a very few globular proteins to investigate scientific and structural aspects (Yu et al., 1994; Agena et al., 1999; Abeln & Frenkel, 2011), modeling of solubility of fibrous protein, based on fully experimental results has not been reported. Considering the dependency of solubility of myosin from freshwater fish *L. rohita* on system parameters (Das & Chattoraj, 1989), the present work is aimed at the regression modeling of those experimental results of myosin-sodium chloride-buffer systems at different ionic strength/ molarity of NaCl, pH and temperature to generate mathematical equations to be used in quality control of fish based fabricated food.

Materials and Methods

Myosin was isolated from dorsal portion of freshwater fish rohu (*Labeo rohita*) weighing 250 to 300 g. After deskinning, deboning, macerating and washing the muscle, myosin was extracted using phosphate buffer (PB) of pH 6.2, ionic strength (μ) 0.1 as solvent containing KCl, $MgCl_2$ and $Na_4P_20_7$. Isolated myosin was in gel form, stored at 4°C and used within 3 days. The gel was used for solubility experiments as briefly outlined below. Details of isolation of myosin followed by solubility experiments are described elsewhere (Das & Chattoraj, 1989).

The isolated myosin gel was suspended in 0.02 M NaCl and 2 ml of this freshly prepared suspension was added to 8 ml NaCl solution of increasing molarities in 50 ml stoppered conical flasks. On shaking isothermally (± 0.5°C) in water bath for 2 h at variable temperature (10-65°C), part of added myosin dissolved. Undissolved part was separated by centrifugation at 5000 rpm for 15 min and the supernatant was filtered through Whatman no. 1 filter paper to remove any trace of suspended material. The absorbance of the supernatant was determined using Folin - Ciocalteau reagent (Lowry et al., 1951). The absorbance was then converted to myosin content with the help of a standard curve plotted as: absorbance of supernatant by Folin reagent *vs.* myosin content in g l^{-1} of the same solutions. To determine the myosin content, nitrogen content of the concerned supernatant was estimated by microkjeldahl method, followed by multiplying the nitrogen content by a conversion factor 6.02 (Baily, 1944; Das & Chattoraj, 1989).

The effect of pH on the solubility of myosin was studied at 27°C by taking 2 g of myosin gel in 10 ml of different buffers (ì 0.1), both in absence and presence of NaCl. The variable pHs were maintained in two ranges, *viz.*, 3.8-5.8 using acetate buffer (AB) and 6.0-7.7 using PB. Soluble myosin content was determined as mentioned above.

Regression equations were developed between the solubility of myosin (S) (dependent variable) and molarity of NaCl/pH/temperature of the system as independent variable, with the help of Microsoft Excel 2000. The general form of the equation attempted is presented below in the form of eq.(1):

$$y = f(x) = b_0 + b_1x + b_2x^2 + b_3x^3 \ldots + b_px^p \quad \ldots (1)$$

where y is dependent variable, b_0, b_1, b_2,.... b_p are regression coefficients and x is independent variable (predictor). Several degree of polynomial (permissible in Excel) equation was attempted. The criteria used to evaluate the goodness of fit of each equation were the co-efficient of determination (R^2), the root mean square error (RMSE) and randomness of the residual plot that is the plot of the differences between predicted and experimental values of dependent variable against the independent variable.RMSE was calculated as follows (Aviara et al., 2006)

$$RMSE = \sqrt{\left[\frac{1}{N}\sum_{i=1}^{N}(S_1 - S_{pi})^2\right]} \quad \ldots (2)$$

Where S_i is the i^{th} experimental solubility value, S_{pi} is the i^{th} predicted solubility value, and N is the number of experimental data.

For each condition, the model which provided R^2 close to 1, RMSE close to 0 and random residual plot was considered to be the best fit.

Results and Discussion

In Fig. 1, the marker represents the experimental results on solubility of myosin from rohu fish in PB (pH 6.2) in presence of various concentrations (molarities) of NaCl at different temperature. The solubility values are comparable to those reported by Chen & Jaczynski (2007) for rainbow trout protein. The equations developed from the data in Fig. 1 are included in Table 1, where M and S represent the molarity of NaCl and solubility of myosin in g l^{-1} respectively; the corresponding trend lines are shown in Fig. 1. It is observed that for 10-33°C, the trend follows polynomial of four degree, but of three degree for subsequent temperatures. From the statistical parameters in Table 1 it may be noted that excepting the R^2 for 65°C, the R^2 for other temperatures and both RMSE and nature of residual plots (not shown) for all the temperatures including those of 65°C substantiate that the developed equations could be used for practical purposes (Aviara et al., 2006). Since all the trendline equations are of single predictor and contain positive and negative regression coefficients, the resultant effect is controlled by the predictor, i.e., molarity of NaCl as well as the numerical value of the coefficients. At 10, 27 and 33°C the coefficients of the term in single power of M are all positive and more than 1 and, therefore, when the value of M is less than 1, the linear term is predominant in predicting the upward rising trend of the plots. When M becomes more than 1, the terms in its higher power become prominent and contribute according to the sign and value of regression coefficients; the comprehensive effect appears to be gradual decrease in solubility. At 45-65°C, in addition to higher power terms, the coefficient of the linear terms are also fractions, all together leading to very low solubility for the whole span of molarity.

At any molarity of NaCl (Fig. 1), solubility is found to increase appreciably when the temperature (T) is decreased from 65 to 10°C. At 10, 27 and 33°C, solubility initially increases with increase in M of NaCl up to about 0.80-0.95, which may be due to

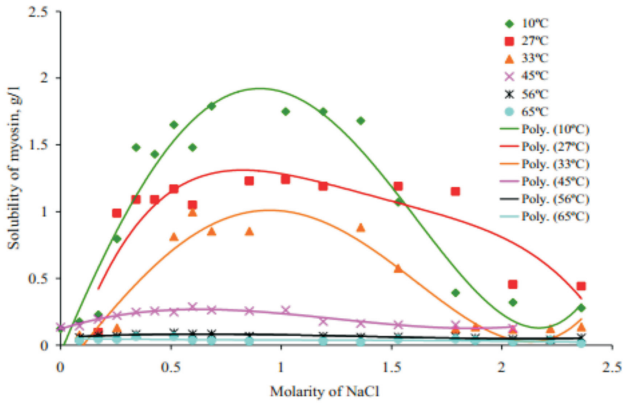

Fig. 1. Solubility of myosin in phosphate buffer (pH 6.2, ionic strength 0.1) containing increasing concentration of sodium chloride at different temperature.

salting-in phenomena. Above this range of NaCl concentration solubility is found to decrease sharply, probably as a result of the well-known salting-out effect. Thus, salting-in region is followed by the salting-out region as has been reported for globular proteins. However, in case of globular proteins, namely hemoglobin and β-lactoglobulin, salting-out phenomenon is usually not observed at high concentrations of the uni-univalent salt sodium chloride (Das & Chattoraj, 1989). These proteins are usually precipitated by $(NH_4)_2SO_4$ or Na_2SO_4. Therefore, behaviour of rod-shaped myosin molecule in this respect is different from that of globular shaped protein such as hemoglobin, â-lactoglobulin, etc. It may also be pointed out here that another rod-shaped protein molecule fibrinogen can be salted out by NaCl (Das & Chattoraj, 1989). At 45°C, the sharp peak zone is almost absent and the small solubility indicates that myosin is denatured. Denaturation at such low temperature may be due to low surface energy because of its rod shape (Das & Chattoraj, 1984). At 56°C or even at 65°C, protein solubility is still observed to some extent although myosin is denatured by thermal energy. In all probability, myosin is somehow fragmented in this range of temperatures and thus may dissolve slightly in the aqueous phase (Harrington & Rodgers, 1984).

The multiple values of root M (molarity of NaCl in the medium) of the developed polynomials for which their first derivative displayed zero value was determined by iterative method (Scarborough, 1966). Among these values of M, the particular value for

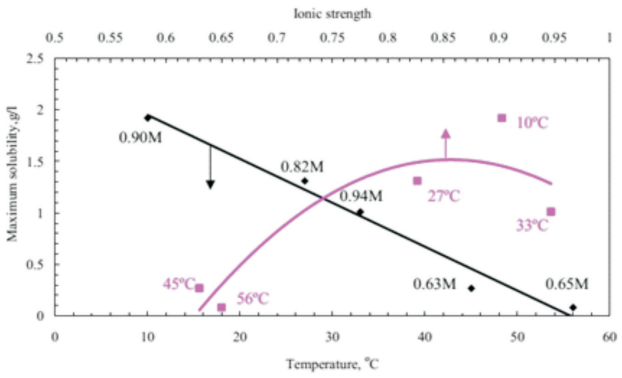

Fig. 2. Maximum solubility of myosin obtainable at different temperature and concentration of sodium chloride. Data levels represent corresponding temperature (pink) and molarity of NaCl (black).

which second derivative of the polynomial indicated negative value was determined. This M is the molarity corresponding to maximum value of S i.e., maximum solubility, S_{max} (g l^{-1}). Values of S_{max} for different systems along with the corresponding M and T are presented in Table 2.

It is observed that M required for S_{max} is dependent on T. For 65°C, no root corresponding to maximum value could be evaluated. The trend lines developed between S_{max} and T as well as S_{max} and corresponding M are shown in Fig. 2; the developed equations and their goodness of fit (Table 2) indicate acceptability of the mathematical models developed. The negative slope of the straight line shows that for the range of M from 0.63 to 0.94, S_{max} decreases linearly with temperature from 10 to 56°C, whereas for 10-56°C, S_{max} exhibits peak value at M of about 0.85.

Fig. 3 shows the experimental results and the corresponding trend lines for solubility (S) of myosin as a function of pH (P) at 27°C, both in presence and absence of NaCl. The model equations developed therefrom along with the error terms evaluated are shown in Table 1. It is observed that three degree polynomial is followed in absence of NaCl, whereas two degree in presence of NaCl. Iterative method of root determination (Scarborough, 1966) indicates minimum solubility at pH 5.74 in absence of NaCl. Since protein is least soluble at isoelectric pH, the isoelectric pH of myosin from this result may be taken as 5.74 in absence of NaCl. It may also be noted that the similarly determined root

Table 1. Modeling for solubility of myosin in different systems

System: NaCl-Buffer (μ 0.1)	T,°C	Equation*	Goodness of fit§	
			R^2	RMSE
NaCl (variable M) -PB (pH 6.2)	10	$S = 0.5389\ M^4 - 1.5379\ M^3 - 0.9693\ M^2 + 3.9232\ M - 0.0563$	0.9275	0.1744
	27	$S = -0.4822\ M^4 + 2.7773\ M^3 - 5.9987\ M^2 + 5.3126\ M - 0.3254$	0.7307	0.1850
	33	$S = 0.4162\ M^4 - 1.3672\ M^3 + 0.1100\ M^2 + 2.0559\ M - 0.2093$	0.9173	0.1043
	45	$S = 0.1512\ M^3 - 0.5642\ M^2 + 0.5295\ M + 0.1203$	0.9141	0.0157
	56	$S = 0.0223\ M^3 - 0.0912\ M^2 + 0.0893\ M + 0.057$	0.7830	0.0065
	65	$S = -0.0046\ M^3 + 0.0153\ M^2 - 0.0221\ M + 0.0496$	0.2777	0.0111
NaCl (0.0 M)-AB (pH 3.8-5.8)/PB (pH 6.0-7.7)	27	$S = -0.3536\ P^3 + 7.0508\ P^2 - 46.007\ P + 98.828$	0.7523	0.4140
NaCl (0.25 M)-AB (pH 3.8-5.8)/PB (pH 6.0-7.7)	27	$S = 0.3171\ P^2 - 2.9567\ P + 6.8214$	0.7914	0.3433
NaCl (0.80 M)-AB (pH 3.8-5.8)/PB (pH 6.0-7.7)	27	$S = 0.3229\ P^2 - 2.6691\ P + 5.4811$	0.9021	0.2920

T, Temperature; *S, M, P are solubility of myosin in g/l, molarity of NaCl, pH of medium; §, All residual plots show random nature

Table 2. Maximum solubility of myosin obtained at different temperature and sodium chloride concentration

T,°C	M	Maximum solubility, S_{max} (g l^{-1})	Equation
10	0.90	1.922	Between S_{max} and T:
27	0.82	1.310	S_{max} = - 0.0426 T + 2.3764
33	0.94	1.009	(R^2 = 0.9761; RMSE = 0.1045; Residual plot = random)
45	0.63	0.268	Between S_{max} and M:
56	0.65	0.082	S_{max} = - 28.512 M^2 + 48.833 M - 19.392 (R^2 = 0.8175; RMSE = 0.2891; Residual plot = random)

T, temperature; M, molarity of NaCl

Table 3. Relation between isoelectric pH (pH_{iso}) and molarity (M) of NaCl at 27 ºC

Molarity of NaCl (M)	Observed isoelectric pH, (pHiso)	Relation between isoelectric pH and molarity of NaCl
0	5.74	pHiso = -1.8403M + 5.4874
0.25	4.66	(R^2 = 0.8426)
0.80	4.13	

P for minimum solubility of myosin is observed at pH 4.66 and 4.13 for 0.25 M and 0.8 M NaCl, respectively (Table 3).

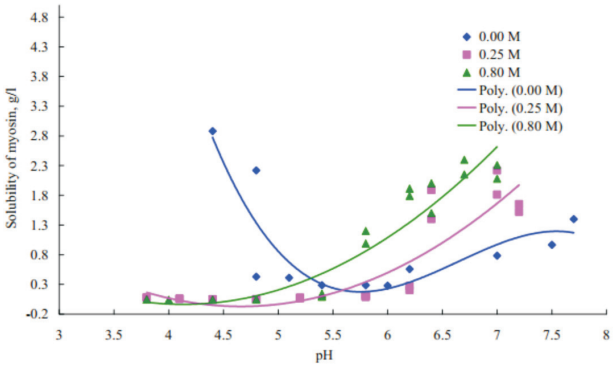

Fig. 3. Solubility of myosin as a function of pH at 27ºC in presence of different molarities of NaCl.

The relation between isoelectric pH and molarity of NaCl is thus found to follow a straight line (shown in Table 3) with negative slope, i.e., isoelectric pH shifts to acidic side in presence of salt. Chen & Jaczynski (2007) also noted shifting towards acid side in presence of salt for rainbow trout protein. It is also interesting to note that in absence of NaCl, solubility increases (Fig.3) on either side of isoelectric pH 5.74, while in presence of NaCl, solubility increases only on the higher pH side. In all probability, in absence of NaCl the electrostatic potential around myosin is high and this may be responsible for its solubility in both acid and alkaline media. In presence of NaCl, the positive electrostatic charge is suppressed considerably, as a result of which protein becomes insoluble below the isoelectric pH. Above pH 5.5, myosin is, however, soluble to a significant extent and the solubility increases with increase of salt concentration in the medium. It may be worth mentioning that in absence of salt, salmon myosin is insoluble at pH 4-5 and isoelectric pH of the muscle is within 4.5-5.5 (Lin & Park, 1998; Huss, 1988).

In conclusion, depending on temperature, effect of sodium chloride concentration on solubility of freshwater rohu fish myosin could be described by polynomial equation with appropriate accuracy. Over the 10-56°C range, highest solubility is obtained at around 0.85 (M) NaCl and extent of maximum solubilization increases linearly with lowering of temperature. Considering pH as the

predictor, solubilization of this protein in absence of NaCl follows three degree polynomial, whereas polynomial of two degree is followed in presence of NaCl. Isoelectric pH *viz.,* the pH resulting in minimum solubility of myosin decreases linearly with increase of molarity of sodium chloride.

Acknowledgments

The author is indebted to Prof. D. K. Chattoraj, retired professor of the Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata-700 032, India, for his guidance for the experimental work.

References

Abeln, S. and Frenkel, D. (2011) Accounting for protein-solvent contacts facilitates design of non-aggregating lattice proteins. Biophys J. 100(3): 693-700

Acton, J. C. (1972) Effect of heat processing on extractability of salt-soluble protein, tissue binding strength and cooking loss in poultry meat loaves. Food Sci. 37: 244-246

Agena, S. M., Pusey, M.L. and Bogle, I. D.L. (1999) Protein solubility modeling. Biotechnol. Bioeng. 64: 144-150

Asghar, A., Morita, J. I., Samejima, K and Yasui, T. (1984) Biochemical and functional characteristics of myosin from red and white muscles of chicken as influenced by nutritional stress. Agric. Biol. Chem. 48: 2217-2224

Aviara, N. A., Ajibola, O. O., Aregbesola, O. A. and Adedeji, M. A. (2006) Moisture sorption isotherms of sorghum malt at 40 and 50°C. J Stored Product Res. 42: 290-301

Baily, K. (1944). The proteins of skeletal muscle. Adv. Protein Chem. 1: 289-317

Chen, Y. C. and Jaczynski, J. (2007) Protein recovery from rainbow trout (Oncorhynchus mykiss) processing byproducts via isoelectric solubilization/precipitation and its gelation properties as affected by functional additives. J. Agric. Food Chem. 55: 9079-9088

Das, M. and Chattoraj, D. K. (1984) Thermodynamics of binding water and solute to myosin. J. Biosci. 6(5): 589-599

Das, M. and Chattoraj, D. K. (1989) Physico-chemical aspects of solubility of myosin in aqueous media. Indian J. Biochem. Biophys. 26: 315-324

Elavarasan, K., Kumar, V. N. and Shamasundar, B. A. (2014) Antioxidant and functional properties of fish protein hydrolysates from freshwater carp (Catla catla) as influenced by the nature of enzyme. J. Food Process. Pres. 38: 1207-1214

Ford, A. L, Jones, P. N, MacFarlane, J. J, Schmidt, G. R. and Turner, R. H. (1978) Binding of meat pieces: objective and subjective assessment of restructured steakettes containing added myosin and/or sarcoplasmic protein. J. Food Sci. 43: 815-818

Geirsdottir, M., Hlynsdottir, H., Thorkelsson, G. and Sigurgisladottir, S. (2007) Solubility and viscosity of Herring (Clupea harengus) proteins as affected by freezing and frozen storage. J. Food Sci. C: Food Chem. Toxicol. 72(7): C376-C380

Harrington, W. F. and Rodgers, M. E. (1984) Myosin. Ann. Rev. Biochem. 53: 35-73

Huss, H. H. (1988) Fresh fish: quality and quality changes - A training manual, FAO Fisheries Series, no. 29, 20 p, Ministry of Fisheries, Technical University, Danish International Developmental Agency, Rome

Keever, B. D. (2011) Salt concentration and species affects protein extractability and processed meat characteristics. M. Sc Thesis. 12 p. University of Illinois at Urbana-Champaign. Urbana, Illinois

Kelleher, S. D., Feng, Y. and Hultin, H. O. (2004) Livingston MB. Role of initial muscle pH on the solubility of fish muscle proteins in water. J. Food Biochem. 28: 279-292

Lin, T. M. and Park, J. W. (1998) Solubility of salmon myosin as affected by conformational changes at various ionic strengths and pH. J. Food Sci. 63 (2): 215-218

Lowry, O. H., Rosebrough, N. J., Farr, L A. and RANDALL, R. J (1951) protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Macfarlane, J. J, Schmidt, G. R. and Turner, R. H. (1977) Binding of meat pieces: a comparison of myosin, actomyosin and sarcoplasmic proteins as binding agents. J. Food Sci. 42: 1603-1605

Miller, A. J, Ackerman, S. A, and Palumbo, S. A. (1980) Effects of frozen storage on functionality of meat for processing. J. Food Sci. 45: 1466-1471

Nakayama, T. and Sato, Y. (1971) Relationship between binding quality of meat and myofibrillar proteins. IV. Contribution of native tropomyosin and actin in myosin B to rheological properties of heat set minced-meat gels. J. Texture Stud. 2(4): 475-488

Nowsad, A. A., Khan, A. H. and Niwa, E. (1999) Five Tropical freshwater fish Species: Setting ability and other characteristics of the minces. Asian Fisheries Sci. 12: 175-186

Park, J. W. (2009) Fish protein isolate and its superior functionality. In: Proceedings of the 62nd Reciprocal Meat Conference, American Meat Science Association, Rogers, Arkansas, 21–24 June, pp 56-63

Peng, I. C., Dayton, W. R., Quass, D. W. and Allen, C. E. (1982) Investigation of soybean 11S protein and myosin interaction by solubility, turbidity and titration studies. J. Food Sci. 47: 1976-1983

Saha, A. Lee, Y., Meullenet, J. F. and Owens, C. M. (2009) Consumer acceptance of broiler breast fillets marinated with varying levels of salt. Poultry Sci. 88: 415–423

Samejima, K., Egelandsdal, B. and Fretheim, K. (1985) Heat gelation properties and protein extractability of beef myofibrils. J. Food Sci. 50: 1540-1543

Samejima, K., Hashimoto, Y., Yasui, T. and Fukazawa T. (1969) Heat gelling properties of myosin, actin, actomyosin and myosin-subunits in a saline model system. J. Food Sci. 34: 242-245

Scarborough, J. B. (1966) Numerical Mathematical Analysis, 6th edn., 297 p, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India

Sen, D. P. (2005) Advances in Fish Processing Technology.70 p, Allied Publishers Pvt. Ltd., New Delhi, India

Shaviklo, G. R. (2008) Evaluation and Utilization of Fish Protein Isolate Products. M. Sc. Thesis. 2 p, University of Iceland, Reykjavík, Iceland

Siegel, D. G. and Schmidt, G. R. (1979) Ionic, pH, and temperature effects on the binding ability of myosin. J. Food Sci. 44: 1686-1689

Stefansson, G. and Hultin, H. O. (1994) On the solubility of cod muscle proteins in water. J. Agric. Food Chem. 42: 2656-2664

Thakur, A. K. (2014) Production and disposal, section A. Handbook on Fisheries Statistics. 12th edn., 5 p, The department of animal husbandry, dairying and fisheries, Fisheries Survey of India, Ministry of Agriculture, Government of India, Mumbai

Thawornchinsombut, S. and Park, J. W. (2004) Role of pH in solubility and conformational changes of pacific whiting muscle proteins. J. Food Biochem. 28: 135-154

Ugalde-Benßtez, V. (2012) Meat emulsions. In: Handbook of meat and meat and meat processing (Hui, Y. H. Ed), 2nd edn., pp 447-456, CRC Press, Boca Raton, FL

Vega-Warner, V. Merkel, R. A. and Smith, D. M. (1999) Composition, solubility and gel properties of salt soluble proteins from two bovine muscle types. Meat Sci. 51: 197-203

Yapar, A., Atay, S., Kayacier, A. and Yetim, H. (2006) Effects of different levels of salt and phosphate on some emulsion attributes of the common carp (Cyprinus carpio L., 1758). Food Hydrocolloid. 20: 825-830

Yathavamoorthi, R., Sankar, T.V. and Ravishankar, C. N. (2012) Effect of ice storage on the characteristics of common carp surimi. Fish. Technol. 49: 38-44

Yeong, W. T., Mohammad, A.W., Anuar, N. and Rahman, R. A. (2002) Potential use of nanofiltration membrane in treatment of wastewater from fish and surimi industries. Songklanakarin J. Sci. Technol. 24 (Suppl.): 977-987

Yu, M., Arons, J. deS. and Smit, J. A. M.(1994) A simple model for estimating protein solubility in aqueous polymer solutions. J. Chem. Technol. Biotechnol. 60: 413-418

Yueh, M. H. (1975) Sea food products, U S Patent 3863017