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Research Paper

Restructured Fish Nuggets from 'Wallago Attu' Extended with Soy Protein Isolate

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ABSTRACT

The present study was conducted to prepare restructured fish nuggets from comparatively low-value cuts of *'Wallago attu'* (Boal), with incorporation of soy protein isolate at different levels viz. 2.5% (T_1), 5% (T_2) and 7.5% (T_3). Physicochemical, microbiological and sensory qualities were estimated on 0, 3, 7 and 14 days of refrigerated storage. Cooking yield (%) and Water Holding Capacity (WHC) were found to be significantly higher ($p<0.05$) in T_3 group (89.31± 1.28 and 45. 72 ±0.79 respectively) in 0 day, when compared to control group which had a cooking yield and WHC of 83.14 ± 0.67 and 40.11 ± 0.76 respectively on 0 day. Moisture content differences between the treatments were significant ($p<0.05$). Highest moisture % observed in T_3 group (82.54 ± 1.51), whereas lowest in control group (75.12 ± 0.76) on 0 day. pH values were not affected ($p>0.05$) by treatment but decreased significantly ($p<0.05$) with the progress of storage. However, beyond 7th day, it showed a significant ($p<0.05$) increasing trend. Thio-barbituric acid (TBA) value, peroxide value, free fatty acids and total plate counts exhibited significant ($p<0.05$) increasing trend with the storage period and significant decreasing trend ($p<0.05$) with increase in level of incorporation. Soy protein isolate up to 7.5% increased the sensory acceptability of the nuggets throughout the storage period. However, within a treatment group, sensory scores deteriorated significantly ($p<0.05$) with the advancement of storage period. Based on the results, nuggets containing 7.5% SPI are recommended for the preparation of cost effective fish nuggets.

Keywords : *Fish, Wallago attu, Restructured, Nuggets, Soy protein*

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INTRODUCTION

Fish is a highly nutritious, tasty and easily digestible food commodity. Fish products are comparable to meat and dairy products in nutritional quality. It is an important dietary constituent of several population groups and it has significant nutritional value, such as high quality proteins, vitamins, minerals and lipids, besides being the largest source of ω-3 series polyunsaturated fatty acids (especially the EPA and DHA), which bring several benefits to human health (Goncalves and Passos 2010). In seafood processing, it is of great interest to maximize the yield of marketable products, including the developments of methods for restructuring low-value cuts and trimmings to improve their appearance, flavour and texture and to enhance the market value. Then an alternative process involves obtaining a fish paste by mechanical separation of the flesh and then preparing restructured fish products with high economic and nutritive value (Ramirez *et al*. 2007). Restructuring yields fish products with high commercial value from different sources: noncommercial fish species, fish with size smaller than commercial (such as shrimp by-catch) and trimmings from nuggets of commercial fish species. Although several methods of restructuring have been developed, the most commonly used include cutting, tumbling and massaging, with or without vacuum (Ramírez *et al.* 2007). However, marine and fresh water products are known to easily deteriorate during processing and storage due to the action of different factors as microbiological development, endogenous enzyme activity, non-enzymatic lipid oxidation and browning (Cheftel and Cheftel 1976).

'Wallago attu' is a species of catfish in the family *Siluridae,* or "sheat fishes". The fish is commonly known by its genus name, Wallago or Lanchi or Boal. It is found in large rivers and lakes in much of the Indian Subcontinent and in parts of Southeast Asia. The species can reach 2.4 m (8 feet) total length. It ranges mainly across India, Nepal, Bangladesh and Sri Lanka, but is also found in Thailand, Cambodia, Vietnam and Malaysia and is also reported from Afghanistan.

Meat or fish extenders are primarily plant proteins from legumes, with soy beans as the major source. Textured Vegetable Protein (TVP) is the most common soy bean extender. These cheaper plant proteins "extend" the more expensive meat or fish proteins, resulting in acceptable overall protein contents of lower cost products. Extenders are added in sizeable amounts that increase the bulk of the products, but this may also alter their quality. From animal protein sources, whole

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milk and eggs can be considered as extenders. Soy protein concentrates are also very useful ingredients in many processed meat products, where they not only enhance the nutritional value but primarily the water binding and fat emulsifying capacity (FAO 2007). Soy bean is a highly nutritious food material that contains well balanced amino acids and desirable fatty acids and it plays an important role as a protein resource for Asian people (Das *et al.* 2008). Recently, many functions of soy beans have been in the spotlight, for example, reducing the risk of heart disease, cancer, and so on (FDA 1999).

A number of studies have been reported on successful incorporation of fish flesh into starch based materials to process and produce nutritionally enriched products. This had in one side enhanced the sensory properties and on the other hand it could develop value added fish meat products. In the present study, attempts have been made to develop a Restructured Fish nugget extended with Isolated Soy Protein (ISP) powder. Physico-chemical properties, storage stability study and sensory evaluation were done to evaluate the quality and acceptability of the product among the consumers. Along with a detailed comparison between the control and treatment nuggets with different level of extenders has been put on record.

MATERIALS AND METHODS

***Raw fish, sampling and mincing*:** Fresh Boal fish (*Wallago attu'*) was obtained directly from the market, thoroughly rinsed with cold tap water, stored in ice and transported to the laboratory immediately in less than one hour, where it was washed, weighed, headed, gutted and filleted manually. Sample preparation, mincing and clarification procedure was done as per the procedure described by Tokur *et al*. 2006. Skin and bones were removed manually and fillets were washed and weighed. Fillets were ground with 4 mm plate to obtain a homogeneous mince. The obtained mince was maintained at refrigeration temperature until the clarification process. Minced fish was washed in ice cold water (2°C) with a ratio of 1.5:2 (mince meat to water), to eliminate a mossy odor, and strained using a cheese cloth in a refrigerator at 2°C for 8 h. Afterwards, the samples were dewatered by squeezing manually. The mince is mixed with spice and condiments to make the batter. The control formulation (C) contained 93.5% fish mince, 1.5% salt, 1% sugar, 3% wheat flour, 0.25% cumin, 0.25% onion, 0.25% garlic powder and 0.25% pepper. In the three treatment groups (T_1, T_2 and T_3 respectively), 2.5%, 5% and 7.5% Isolated Soy Protein (ISP) powder replaced the minced fish meat. The batter was mixed well and then it was put into a Phillips kitchen blender with a cold water/batter flour ratio of 2.2:1 (w/w) for 2 min. The clarified minced was examined to make sure that it was free of bones and then "glued" together using a food-grade binder – sodium alginate @ 0.5%.

Preparation and cooking of restructured fish meat nuggets: Four groups of moulds were prepared by stuffing the meat batter into a moulder (6×4×2 inch) which was squeezed with wooden press and pressure cooked so that internal temperature at the geometrical centre of the mold will be 85°C, when measured by a probe thermometer. Now the cooked fish blocks were cooled to room temperature and sliced into nuggets and packed in UV sterilized low density polyethylene bags and analyzed for study of different parameters.

Analysis: Analysis for determination of proximate composition, initial chemical quality and initial microbial load, sensory evaluation etc. were performed on the production day i.e. 0^{th} day. Thereafter chemical deterioration and microbiological quality estimation were repeated on 3^{rd}, 7^{th} and 14^{th} day, in refrigerated (4±1°C) storage. All the parameters were performed in triplicate and data obtained were carried out using ANOVA technique according to the method described by Snedecor and Cochran (1989) by Completely Randomized Design (CRD) for all parameters except storage parameters for which Randomized Block Design (RBD) was followed.

***Cooking yield*:** Nuggets were weighed before and after cooking and cooking yields were determined by using the following formula –

$$\text{Percent cooking yield} = \frac{\text{Cooked weight in g}}{\text{Raw weight in g}} \times 100$$

***Water holding capacity* (WHC):** Water Holding Capacity (WHC) was determined by modifying the method of Hughes *et al*. (1997) as outlined by Cengiz *et al.* (2007).

Proximate analysis: Moisture, protein, fat and total ash content of the nugget sampleswere determined as per AOAC (1984).

pH: pH of the cooked nugget samples were estimated on each storage days by the method described by Egbert *et al*.(1992).

Thiobarbituric acid (TBA) value: Thio-Barbituric acid value was estimated as per procedure given by Tarladgis *et al.* (1960).

Peroxide value: Peroxide value was estimated as per procedure given by AOCS (1992) with slight modifications. 5 g of sample was weighed and mixed with 30 ml acetic acid-chloroform

solution (3:2) in 250 ml glass-stoppered Erlenmeyer flask. Slurry obtained was gently swirled to extract lipid and then 0.5 mL saturated potassium iodide solution was added. After reaction for 1 minute with occasional shaking, 30 ml of distilled water and 0.5 ml of 0.5% starch solution were added. The mixed solution was titrated with 0.01 N sodium thiosulphate until intense blue colour disappeared. A blank was also determined and subtracted from sample titration.

$$PV\ (mEq/Kg) = \frac{\text{ml of Na-thiosulphate x N of Na-thiosulphate}}{\text{Weight of sample}} x10$$

Free fatty acid: Free fatty acid value was determined by modified AOCS method (Koniecko 1979).

Microbiological analysis

Preparation of samples: Samples were prepared according to APHA (1992). 1 g of sample was transferred to 99 ml of normal saline solution and dilutions were prepared.

Total plate count: It was determined using plate count agar as detailed in APHA (1992). One ml of appropriate dilution of sample was transferred aseptically to sterile petri-plates in triplicate. The plates were then poured with 10-15 ml melted agar medium at 45°C. After solidification the petri-plates were incubated at 37°C for 24-28 hrs. The colonies were counted by using colony counter. The average number of colonies was multiplied with dilution factor to obtain total count as Colony Forming Unit (CFU) per gm of the sample and expressed as log CFU/g.

Sensory evaluation: The cooked nugget samples were served warm to panelists for sensory evaluation on 0^{th}, 3^{rd}, 7^{th} and 14^{th} day. Sensory evaluation method using an eight-point descriptive scale (Keeton 1983) was followed with modifications, where 8 = excellent; 1 = extremely poor. The sensory panel consisted of seven experienced scientists, faculties and post-graduate students of the department. The panelists were explained about the nature of experiments without disclosing the identity of samples and were asked to rate their preferences on 8-point descriptive scale on the sensory evaluation proforma for different traits. Samples were warmed using microwave oven for 1 min, cut across the centre to make of equal size and shape and served to panelists. Water was provided to rinse mouth between the samples. The panelists judged the samples for their general appearance, flavor, juiciness, texture and overall acceptability.

***Statistical analysis*:** Statistical analysis of the data obtained was carried out using 4 (treatment) × 6 (replications) randomized block design. All chemical and physical determinations were in triplicate. Data were subjected to one-way analysis of variance. The storage data were analyzed on the basis of 4 (treatments) × 4 (storage days) × 6 (replications) with two-way analysis of variance. Further to test the significance among each group, Post-hoc test (Tukey's HSD) has been carried out by SPSS- 16® software package at 5% level of significance.

RESULTS AND DISCUSSION

Cooking yield: The product yield was comparable between control and treated fish nuggets. Mean ± SE of cooking yield of fish nuggets are presented in Table 1. It is evident from the results that cooking yield of control and soy protein powder treated fish nuggets were significantly decreased ($p<0.05$) with the advancement of storage period. Maximum cooking yield was noticed in T_3 group in all storage days.

Process economics are better if product cook yield is high. Also, meat products with a high cook yield tend to be more juicy and tender than products with a low cook yield (Swan *et al*. 1998). The findings of this study are in congruence with the findings of Shabanpour and Jamshidi (2013), who also found the product yield of fish nuggets in the range of 85.07±2.07 to 89.34 ±0.77. An increase in cooking yield of fish fillets has been also noticed by Arason *et al.* (2009), after addition of Fish Protein Isolate (FPI), which again supports the findings of this study. Chilled and frozen cod fillets resulted in higher total yield compared with control fillets and salt injected fillets (Arason *et al.* 2009).

***Water holding capacity* (WHC):** Mean ± SE of WHC (%) of fish nuggets are presented in Table 1. It has been noticed that up to 3^{rd} day of storage, there was no significant change in water holding capacity, but thereafter it decreased significantly ($p<0.05$), although the trend was always decreasing. Maximum water holding capacity was found in T_3 group, whereas minimum for control group. Fish cakes made by substituting protein powders part by potato flour resulted that potato flour plays important roles for colour, texture and WHC of the final products. Compared to Fish Protein Hydrosylate (FPH) powders addition of potato flour gave lighter and firmer fish cakes with very high water holding capacity (Arason *et al.* 2009). All of the cryoprotectants tested were effective in increasing the water holding capacity of the fish minces. Sodium caseinate was the most effective but resulted in very viscous mixtures which were difficult to handle (Brennan and Gormley 1999). The strength of fish gels is a useful indicator of the suitability of fish species for use in fish products (Maier *et al*. 1997). Anese and Gormley (1996) found that ingredients that increased fish gel strength were those that reduced water holding capacity of the fish mince.

Table 1: Mean ± SE values of cooking determinants of cooked Fish Nuggets stored at refrigerated temperature (4±1°C) for different storage period (n=6)

Parameters	Storage days	Control	T_1	T_2	T_3
Cooking Yield %	0	$83.14^{ax} \pm 0.67$	$84.23^{abx} \pm 0.84$	$87.33^{bcx} \pm 2.11$	$89.31^{cdx} \pm 1.28$
	3	$81.36^{axy} \pm 1.28$	$83.33^{abxy} \pm 1.14$	$85.48^{bcxy} \pm 1.53$	$87.21^{cdxy} \pm 1.71$
	7	$76.37^{ayz} \pm 1.41$	$79.39^{abyz} \pm 1.61$	$82.11^{bcyz} \pm 1.06$	$84.51^{cdyz} \pm 0.87$
	14	$71.87^{az} \pm 1.66$	$73.28^{abz} \pm 1.38$	$77.22^{bcz} \pm 1.25$	$80.05^{cdz} \pm 1.11$
WHC %	0	$40.11^{ax} \pm 0.76$	$42.22^{ax} \pm 1.22$	$43.18^{abx} \pm 0.91$	$45.72^{bx} \pm 0.79$
	3	$39.11^{ax} \pm 0.21$	$42.18^{ax} \pm 1.03$	$42.17^{abx} \pm 0.95$	$44.87^{bx} \pm 1.26$
	7	$38.52^{axy} \pm 0.66$	$41.12^{axy} \pm 1.27$	$41.03^{abxy} \pm 1.25$	$43.77^{bxy} \pm 1.91$
	14	$36.72^{ay} \pm 0.89$	$39.24^{ay} \pm 1.14$	$40.21^{aby} \pm 1.24$	$41.86^{by} \pm 1.61$

*Means bearing different superscripts (a, b, c, d, e) row-wise differ significantly ($p<0.05$).
*Means bearing different superscripts (x, y, z) column-wise differ significantly ($p<0.05$).

Proximate composition:

***Moisture*:** Mean values for moisture (%) of cooked fish nuggets are presented in Table 2. Moisture content of fish nuggets were seen to be enhanced ($p<0.05$) by the addition of soy protein, although 2.5% addition had no significant ($p>0.05$) effect on moisture content. This also can be explained by high water holding capacity of treatment group than the control one. Again, moisture content did not show any significant change ($p>0.05$) with advancement of storage days, although the numerical values reduced to an extent. It might be explained by the fact that there always is some loss of moisture escape of water content as 'drip' in refrigerated storage.

In addition through osmosis process, salt can enhance the meat flavor that causes exit of some moisture and reduces water activities. This water exit also will result in limited bacterial growth and less enzymes activities (Shabanpour and Jamshidi 2013). Asgharzadeh *et al.*(2010) in one of their study on chemical changes in silver carp (*Hypophthalmichthys molitrix*) minced muscle during frozen storage, estimated the moisture content of washed and unwashed minced fish muscle without cryoprotectant mixture 82.4% and 78.6% respectively, which is close to the findings of the present study. Tokur *et al*. (2006) found similar findings in fish fingers produced from washed mince (WF), the moisture, crude protein, lipid and crude ash contents were found to be 70.23%, 10.8%, 2.14% and 1.80%, respectively. Application of CMC in batter increased the moisture content of the nuggets significantly ($p<0.05$), which is associated to the ability of CMC to form gel and retain water during frying (Haghshenas *et al.* 2014).

Table 2: Mean ± S.E. of proximate composition of cooked nuggets under refrigeration storage (4 ±1) °C

	Storage days	Control	T_1	T_2	T_3
Moisture %	0th Day	$75.12^{ax} \pm 0.76$	$77.16^{ax} \pm 0.84$	$79.25^{abx} \pm 1.23$	$82.54^{bx} \pm 1.51$
	3rd Day	$74.22^{ax} \pm 1.26$	$76.05^{ax} \pm 1.31$	$78.22^{abx} \pm 1.63$	$80.97^{bx} \pm 1.26$
	7th Day	$73.21^{ax} \pm 1.21$	$75.72^{ax} \pm 0.89$	$77.22^{abx} \pm 1.09$	$79.06^{bx} \pm 1.01$
	14th Day	$72.12^{ax} \pm 1.28$	$74.21^{ax} \pm 1.48$	$75.88^{abx} \pm 2.15$	$77.55^{bx} \pm 1.94$
Protein %	0th Day	$18.28^{ax} \pm 0.47$	$17.21^{ax} \pm 1.02$	$16.83^{ax} \pm 1.54$	$16.36^{ax} \pm 1.11$
	3rd Day	$18.34^{ax} \pm 1.52$	$17.28^{ax} \pm 1.19$	$16.89^{ax} \pm 0.85$	$16.41^{ax} \pm 0.79$
	7th Day	$18.46^{ax} \pm 0.67$	$17.32^{ax} \pm 0.94$	$16.88^{ax} \pm 0.73$	$16.53^{ax} \pm 1.21$
	14th Day	$18.47^{ax} \pm 0.75$	$17.36^{ax} \pm 0.65$	$16.93^{ax} \pm 0.45$	$16.61^{ax} \pm 1.04$
Ether Extract%	0th Day	$3.56^{ax} \pm 0.11$	$3.12^{ax} \pm 0.09$	$3.03^{abx} \pm 0.14$	$2.92^{bx} \pm 0.11$
	3rd Day	$3.57^{ax} \pm 0.06$	$3.16^{ax} \pm 0.03$	$3.07^{abx} \pm 0.15$	$2.96^{bx} \pm 0.09$
	7th Day	$3.59^{ax} \pm 0.07$	$3.21^{ax} \pm 0.04$	$3.16^{abx} \pm 0.03$	$3.06^{bx} \pm 0.11$
	14th Day	$3.66^{ax} \pm 0.05$	$3.32^{ax} \pm 0.05$	$3.25^{abx} \pm 0.04$	$3.09^{bx} \pm 0.04$
Total Ash %	0th Day	$1.87^{ax} \pm 0.07$	$1.94^{ax} \pm 0.02$	$1.81^{ax} \pm 0.04$	$1.86^{ax} \pm 0.01$
	3rd Day	$1.78^{ax} \pm 0.02$	$1.84^{ax} \pm 0.09$	$1.87^{ax} \pm 0.05$	$1.88^{ax} \pm 0.09$
	7th Day	$1.84^{ax} \pm 0.07$	$1.85^{ax} \pm 0.04$	$1.76^{ax} \pm 0.03$	$1.75^{ax} \pm 0.01$
	14th Day	$1.79^{ax} \pm 0.05$	$1.79^{ax} \pm 0.05$	$1.92^{ax} \pm 0.05$	$1.79^{ax} \pm 0.04$

*Means bearing different superscripts (x, y, z) column-wise differ significantly ($p<0.05$)

Protein: It has been observed there that virtually protein content is unaffected either by the addition of soy protein powder or by the storage period (Table 2). This may be explained by the comparable protein content of 'soy protein powder' with the fish meat.

Goat meat nuggets were prepared using commercially available textured soy granules and reduced beany flavour full-fat soy paste (FFSP) made by simple processing technology to compare the performance of these proteins in a comminuted meat system (Das *et al.* 2008). The findings of this study are in congruence of the findings of Gonçalves and Passos(2010). Using fish proteins as ingredients in processing lines for whitefish generally improved the final products. The improvements were mainly in the form of lower drip loss during storage, higher cooking yield and increased protein content (Arason *et al.* 2009).

Ether extract: Although the decrease in ether extract content with increase in level of incorporation of 'soy protein powder' was noticeable, yet it was not significant ($p>0.05$) up to 5 % level of incorporation (Table 2). However, T_3 group showed a significant ($p<0.05$) reduction in ether extract content when compared to control or T_1 group. With the advancement of storage days, the numerical values increased in all the groups but it remained insignificant ($p>0.05$). It may be due to relative decrease in moisture content as storage period progresses, as it is assumed that moisture and ether extract content in any meat matrix system, is inversely proportional to each other. Fat uptake of fish cakes varies depended on powder added and can depend on emulsification properties of hydrolysates. Fat absorption of the fish cakes was shown to be related to the emulsifying properties of the added powder (Arason *et al.* 2009). The fat contents in fish nuggets in all the treatments groups in this study are comparable to the findings of Shabanpour and Jamshidi (2013).

Total ash: It is evident from the results that, total ash content was not affected ($p>0.05$) by the level of incorporation or by the progress in storage period (Table 2). The ash content varied from 1.9% to 3.7% and these values are related to the curing salts, NaCl and other additives, ingredients of the nuggets formulation (Silva *et al.* 1994).

Storage study

pH: pH of the cooked fish nuggets were decreased significantly ($p<0.05$) up to 7th day but thereafter increased on 14th day of storage. But this trend was somehow not observed in T_3 group (Table 3).The pH values of control and all the treatment groups are comparable to the findings of Raja *et al*. (2014). Mean values of pH of curls showed significantly ($p<0.05$) decreasing trend with increasing days of storage (6.34 ± 0.01 on day 0 and 5.90 ± 0.005 on day 28 for control samples, 6.41 ± 0.009 on day 0 and 6.11 ± 0.02 on day 28 for corn flour incorporated samples, 6.36 ± 0.01 on day 0 and 6.14 ± 0.01 on day 28 for black gram flour incorporated samples, 6.57 ± 0.007 on day 0 and 6.34 ± 0.01 on day 28 for peanut flour incorporated samples). No significant differences during frozen storage were observed by Amzad *et al.*(2011), in the third and sixth months period among Control, Citric acid and Ascorbic acid treated samples. The increment in pH values from 14th day onwards significantly ($p<0.05$) is supported by the previous research carried out by Debbarma and Majumdar (2013), who also postulated that, the pH of mince (6.59) was found to increase slightly after wash (6.93) and thereafter slowly increased to 7.51 on 90th day of storage. Decomposition products such as volatile bases could lead to a pH rise during storage of fish mince. Bennour *et al.* (1991) reported less than one unit increase in pH of mackerel (*Scomber scombrus*) during storage in ice.

Thiobarbituric acid value: It has been noticed from the Table 3 that TBA value of cooked fish nuggets increased significantly ($p<0.05$) with the progress of storage period irrespective of treatment group. Incorporation of soy protein powder into restructured fish nuggets significantly reduced ($p<0.05$) the TBA content of the nuggets especially beyond 3rd day of storage. However, on 0th day, effect of addition of soy protein powder on TBA value was not significant ($p>0.05$).Rancidity development was measured by means of secondary lipid oxidation compound formation (TBA). Scott *et al.* (1992) reported that TBA value above 3-4 mg malonaldehyde kg-1 indicates quality loss in the product. The increment of TBA value in refrigerated as well as frozen storage has been reported by Das *et al.*(2008). But the values of TBA value remained lower than the acceptable level for rancidity (1.0 mg/kg) (Das *et al.* 2008). Off-flavour was not detected by the taste panelists during sensory evaluation of the nuggets.

Peroxide value: Table 3 suggests that, effect of storage and incorporation both have had a significant ($p<0.05$) correlation with the peroxide value content (Mean ± SE) of cooked fish nuggets. Increase in level of incorporation significantly decreased the peroxide value of the nuggets. Up to 3rd day of storage, the increase in peroxide value was not significant ($p>0.05$), but thereafter increased significantly ($p<0.05$).

Starting raw fish showed a low Peroxide value (1.4±0.1) that remained unchanged after the washing process and the freezing step. However, a marked peroxide content increase

could be observed for both kinds of minced materials at the end of the frozen storage. This could be explained as a result of the presence of pro-oxidant enzymes (lipoxygenases, peroxidases, and so on) and chemical pro-oxidant molecules (namely, hemoproteins and metal ions) (Erickson 1997).

Free fatty acid: Free fatty acids are the products of enzymatic or microbial degradation of lipids. Determination of FFA gives information about stability of fat during storage (Das *et al.* 2008). It is evident from the Table 3 that Mean ± SE of free fatty acid (as % oleic acid) increased throughout the storage period in all the treatment groups, although the initial increment up to 3rd day was not found to be significant ($p>0.05$). Incorporation of soy protein powder in different levels into fish nuggets significantly ($p<0.05$) reduced the free fatty acid value in all the treatment groups. This might be attributable to the lower fish meat content in the treatment groups' formulation (T_1, T_2 and T_3) and lower or virtually absence of fatty acid in replacing soy protein powder. Increase in FFA values of various meat and fish products has been reported by Asgharzadeh *et al.*(2010).

Table 3: Mean ± S.E. of Storage study parameters of cooked nuggets under refrigeration storage (4±1) °C

	Storage days	Control	T_1	T_2	T_3
pH	0th Day	$7.21^{ax} \pm 0.08$	$7.12^{axy} \pm 0.05$	$7.15^{ax} \pm 0.16$	$7.17^{ax} \pm 0.11$
	3rd Day	$7.16^{ax} \pm 1.07$	$7.01^{ax} \pm 0.94$	$7.04^{ax} \pm 1.06$	$7.04^{ax} \pm 0.25$
	7th Day	$6.89^{ay} \pm 0.08$	$7.08^{axy} \pm 0.18$	$6.82^{ay} \pm 0.86$	$7.03^{ax} \pm 0.78$
	14th Day	$7.27^{ax} \pm 0.11$	$7.26^{ay} \pm 0.33$	$7.12^{ax} \pm 0.81$	$7.29^{ax} \pm 0.82$
TBA value	0th Day	$0.146^{ax} \pm 0.005$	$0.135^{ax} \pm 0.015$	$0.128^{ax} \pm 0.012$	$0.122^{ax} \pm 0.021$
(mg malonaldehyde kg^{-1})	3rd Day	$0.284^{ax} \pm 0.009$	$0.254^{bx} \pm 0.008$	$0.263^{abx} \pm 0.018$	$0.248^{bx} \pm 0.013$
	7th Day	$0.634^{ay} \pm 0.012$	$0.612^{aby} \pm 0.019$	$0.601^{by} \pm 0.012$	$0.597^{by} \pm 0.015$
	14th Day	$1.853^{az} \pm 0.017$	$1.432^{abz} \pm 0.044$	$1.403^{bz} \pm 0.032$	$1.392^{bz} \pm 0.062$
Peroxide Value (mEq / kg)	0th Day	$1.36^{ax} \pm 0.12$	$1.12^{abx} \pm 0.09$	$1.03^{bx} \pm 0.14$	$1.02^{bx} \pm 0.11$
	3rd Day	$1.63^{axy} \pm 0.08$	$1.26^{bx} \pm 0.03$	$1.37^{abx} \pm 0.15$	$1.26^{bx} \pm 0.09$
	7th Day	$3.51^{ay} \pm 0.05$	$3.36^{aby} \pm 0.04$	$2.96^{by} \pm 0.03$	$2.76^{by} \pm 0.11$
	14th Day	$4.26^{az} \pm 0.05$	$4.12^{abz} \pm 0.05$	$3.85^{bcz} \pm 0.04$	$3.79^{cz} \pm 0.04$
Free Fatty Acid	0th Day	$0.217^{ax} \pm 0.07$	$0.207^{bx} \pm 0.02$	$0.211^{abx} \pm 0.04$	$0.202^{bx} \pm 0.01$
(as % Oleic acid)	3rd Day	$0.423^{ax} \pm 0.02$	$0.314^{bx} \pm 0.09$	$0.307^{bx} \pm 0.05$	$0.308^{bx} \pm 0.09$
	7th Day	$1.842^{ay} \pm 0.07$	$1.315^{by} \pm 0.04$	$1.316^{by} \pm 0.03$	$1.305^{by} \pm 0.01$
	14th Day	$3.79^{az} \pm 0.05$	$1.79^{bz} \pm 0.05$	$1.92^{bz} \pm 0.05$	$1.79^{bz} \pm 0.04$
Total Plate Count	0th Day	$1.32^{ax} \pm 0.032$	$1.24^{abx} \pm 0.021$	$1.23^{abx} \pm 0.047$	$1.18^{bx} \pm 0.022$
(log CFU/g)	3rd Day	$1.75^{ax} \pm 0.073$	$1.55^{bx} \pm 0.073$	$1.61^{abx} \pm 0.047$	$1.58^{bx} \pm 0.036$
	7th Day	$2.54^{aby} \pm 0.051$	$2.46^{ay} \pm 0.048$	$2.45^{ay} \pm 0.033$	$2.61^{by} \pm 0.028$
	14th Day	$4.21^{az} \pm 0.072$	$4.16^{abz} \pm 0.046$	$4.03^{bz} \pm 0.066$	$4.19^{az} \pm 0.061$

*Means bearing different superscripts (a, b, c, d, e) row-wise differ significantly ($p<0.05$).
*Means bearing different superscripts (x, y, z) column-wise differ significantly ($p<0.05$).

Total plate count: Up to 3rd day of refrigerated storage, no significant difference ($p>0.05$) found in total plate count of the samples, but thereafter it increased significantly ($p<0.05$) (Table 3). Again when incorporation level was concerned, significant difference ($p<0.05$) has been noticed beyond the 5% level. This may be due to the relative low proportion of ground fish meat in the formulation of T_3 when compared with control, T_1 and T_2. Similar findings were observed by Raja *et al.* (2014), who postulated that Total plate count (TPC) followed a significantly ($p<0.05$) increasing trend as the storage days progressed in control as well as treated curls. However, total plate counts showed a non-significant ($p>0.05$) difference among different treatments at all the intervals of storage. This study corroborates with the results of Chidanandaiah *et al.* (2009).

Sensory evaluation: General appearance, flavor, juiciness, texture and overall acceptability of the fish nuggets were scored and data obtained were analyzed (Table 4). It has been noticed that scores for all the sensory attributes of control samples were deteriorated significantly ($p<0.05$) beyond the 7th day of storage. Similar decreasing trend was noticed by Raja *et al.* 2014. However, soy protein powder treated nuggets showed more deleterious deterioration in sensory qualities as the storage time progressed. Up to 2.5% incorporation of soy protein powder did not affect the sensory qualities

Table 4: Mean ± S.E. of sensory parameters of cooked nuggets under refrigeration storage (4 ±1) °C

	Storage days	Control	T_1	T_2	T_3
Appearance	0th Day	$6.31^{ax} \pm 0.05$	$6.48^{ax} \pm 0.05$	$6.95^{bx} \pm 0.16$	$6.87^{bx} \pm 0.11$
	3rd Day	$6.26^{ax} \pm 0.11$	$6.31^{abx} \pm 0.14$	$6.54^{by} \pm 0.16$	$6.74^{cx} \pm 1.05$
	7th Day	$6.19^{axy} \pm 0.08$	$6.18^{ay} \pm 0.18$	$6.32^{abyz} \pm 0.26$	$6.53^{by} \pm 0.28$
	14th Day	$6.11^{ay} \pm 0.14$	$6.16^{axy} \pm 0.13$	$6.22^{abz} \pm 0.21$	$6.39^{bz} \pm 0.12$
Flavor	0th Day	$6.45^{ax} \pm 0.08$	$6.52^{ax} \pm 0.05$	$6.82^{bx} \pm 0.16$	$6.77^{bx} \pm 0.11$
	3rd Day	$6.37^{ax} \pm 0.07$	$6.49^{abx} \pm 0.94$	$6.64^{by} \pm 1.06$	$6.84^{cx} \pm 0.25$
	7th Day	$6.23^{axy} \pm 0.08$	$6.18^{ay} \pm 0.18$	$6.38^{byz} \pm 0.26$	$6.51^{cy} \pm 0.18$
	14th Day	$6.15^{ay} \pm 0.11$	$6.16^{ay} \pm 0.33$	$6.22^{abz} \pm 0.81$	$6.29^{bz} \pm 0.82$
Juiciness	0th Day	$6.31^{ax} \pm 0.08$	$6.48^{ax} \pm 0.05$	$6.95^{bx} \pm 0.16$	$6.87^{bx} \pm 0.11$
	3rd Day	$6.26^{ax} \pm 1.07$	$6.31^{abx} \pm 0.94$	$6.54^{by} \pm 1.06$	$6.74^{cx} \pm 0.25$
	7th Day	$6.19^{axy} \pm 0.08$	$6.18^{ay} \pm 0.18$	$6.32^{abyz} \pm 0.86$	$6.53^{by} \pm 0.78$
	14th Day	$6.11^{ay} \pm 0.11$	$6.16^{axy} \pm 0.33$	$6.22^{abz} \pm 0.81$	$6.39^{bz} \pm 0.82$
Texture	0th Day	$6.31^{ax} \pm 0.08$	$6.48^{ax} \pm 0.05$	$6.95^{bx} \pm 0.16$	$6.87^{bx} \pm 0.11$
	3rd Day	$6.26^{ax} \pm 1.07$	$6.31^{abx} \pm 0.94$	$6.54^{by} \pm 1.06$	$6.74^{cx} \pm 0.25$
	7th Day	$6.19^{axy} \pm 0.08$	$6.18^{ay} \pm 0.18$	$6.32^{abyz} \pm 0.86$	$6.53^{by} \pm 0.78$
	14th Day	$6.11^{ay} \pm 0.11$	$6.16^{axy} \pm 0.33$	$6.22^{abz} \pm 0.81$	$6.39^{bz} \pm 0.82$
Overall Acceptability	0th Day	$6.31^{ax} \pm 0.08$	$6.48^{ax} \pm 0.05$	$6.95^{bx} \pm 0.16$	$6.87^{bx} \pm 0.11$
	3rd Day	$6.26^{ax} \pm 1.07$	$6.31^{abx} \pm 0.94$	$6.54^{by} \pm 1.06$	$6.74^{cx} \pm 0.25$
	7th Day	$6.19^{axy} \pm 0.08$	$6.18^{ay} \pm 0.18$	$6.32^{abyz} \pm 0.86$	$6.53^{by} \pm 0.78$
	14th Day	$6.11^{ay} \pm 0.11$	$6.16^{axy} \pm 0.33$	$6.22^{abz} \pm 0.81$	$6.39^{bz} \pm 0.82$

*Means bearing different superscripts (a, b, c, d, e) row-wise differ significantly ($p<0.05$).
*Means bearing different superscripts (x, y, z) column-wise differ significantly ($p<0.05$).

significantly ($p>0.05$), but 5 % and 7.5% level of incorporation had significant difference ($p<0.05$) from control and T_1 sample. A gradual decline of flavour might be due to the expected loss of volatile flavour components from spices and condiments on storage (Das *et al.* 2008). A significant water loss from the fish meat matrix model system during refrigerated storage may be the probable cause of decline in juiciness and texture of the product.

CONCLUSION

Restructured fish nuggets made from comparatively lower value cuts of *'Wallago attu'* fish by flaking, tearing, grinding and forming of nuggets, along with a maximum level of 7.5% soy protein powder incorporation, can be successfully prepared in household as well as commercial condition as a value added fish product. The treated nugget groups showed a high cooking yield and high water holding capacity, having a comparable even better nutritional composition, stable for a longer period of time when compared to the control one. Sensory properties of treated nuggets were not adversely affected even by the maximum level of incorporation; rather it was liked by the panelists. Hence, soy protein powder has a great potential use to formulate emulsion type fish products with good acceptability and cost benefit.

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REFERENCES

Anese M, Gormley R (1996) Effects of dairy ingredients on some chemical, physico-chemical and functional properties of minced fish during freezing and frozen storage. Lebensmittel -Wissenschaft Technologie 29: 151- 157

AOAC (1984) Official Methods of Analysis. 16th edn. Association of official analytical chemists, Will behington, D.C

AOCS (1992) Peroxide value acetic acid-chloroform method. In official methods and recommended practices of the American oil chemists: champaign, IL, 1998a:Cd 8-53

Amzad HR, Shabanpour B, Kashaninejad M, Shabani A (2011) Antioxidative activity of citric and ascorbic acids and their preventive effect on lipid oxidation in frozen Persian sturgeon fillets. Latin Am Appl Res 41:135-140

APHA (1992) Compendium of methods for methods for the microbiological examination of foods 2nd edn. American Public Health Association, Will behington, D.C

Arason S, Karlsdottir M, Valsdottir V, Slizyte R, Rustad T, Falch E, Eysturskard J, Jakobsen G (2009) Maximum resource utilization – Value added fish by-products. Nordic Innovation Centre project number: 04275. Nordic Innovation Centre; Stensberggata 25, NO-0170, Oslo Norway

Asgharzadeh A, Shabanpour B, Aubourgb SP, Hosseini H (2010) Chemical changes in silver carp (*Hypophthalmichthys molitrix*) minced muscle during frozen storage: Effect of a previous washing process. Grasas-Y-Aceites, 61 (1): 95-101

Bennour M, Marrakchi AE, Bouchriti N, Hamama A (1991) Chemical and microbiological assessments of mackerel (*Scomber scombrus*) stored mince. J Food Prot 54: 789-792

Brennan MH, Gormley TR (1999) The Quality of under-utilized deep-water fish species. Final Report; Project Armis No.4560; Agriculture and Food Development Authority. The National Food Centre; Research Report No.-22. Dunsinea, Castleknock, Dublin 15

Cengiz E, Gokoglu N (2007) Effects of fat reduction and fat replacer addition on some quality characteristics of frankfurter-type sausages. Int J Food Sci Technol 42: 366-372

Cheftel J, Cheftel H (1976) Introducción a la Bioquímica y Tecnología de Alimentos. Zaragoza, Spain: Editorial Acribia Pp. 237-323

Chidanandaiah, Keshri RC, Sanyal MK (2009) Effect of sodium alginate coating with preservatives on the quality of meat patties during refrigerated storage. J Muscle Foods 20: 275–292

Das AK, Anjaneyulu ASR, Gadekar YP, Singh RP, Pragati H (2008) Effect of full-fat soy paste and textured soy granules on quality and shelf-life of goat meat nuggets in frozen storage. Meat Sci 80: 607–614

Debbarma S, Majumdar RK (2013) Biochemical and organoleptic changes of surimi from the Thai pangas (*Pangasianodon hypophthalmus*) during frozen storage. Indian J Fish 60(4) : 99-106

Egbert WR, Huffman DL, Chen CM, Jones WR (1992) Microbial and oxidative changes in low fat ground beef during simulated retail distribution. J Food Sci 57(6): 1269-1274, 1293

Erickson M (1997) Lipid oxidation: Flavor and nutritional quality deterioration in frozen foods in Erickson M, Hung Y (Eds.) Quality in frozen food, Chapman and Hall, New York, USA Pp- 141-173

FAO (2007) Meat processing technology for small to medium scale producers. RAP Publication 2007/20. Food and agriculture organization of the United Nations regional office for Asia and the Pacific; Bangkok Pp-14

FDA (Food and Drug Administration) (1999) Food Labeling: Health claims; soy protein and coronary heart disease. Final rule. Federal Regulation 64(206): 57700–57733

Haghshenas M, Hosseinil H, Nayebzadeh K, Khanghah AM, Kakesh BS, Fonood RK (2014) Production of prebiotic functional shrimp nuggets using ß-Glucan and reduction of oil absorption by carboxymethyl cellulose: Impacts on sensory and physical properties. J Aquac Res Development 5:4

Gonçalves AA, Passos MG (2010) Restructured fish product from White Croacker (*Micropogonias furnieri*) mince using microbial transglutaminase. Brazilian Arch Biol Technol 53(4): 987-995

Hughes E, Cofrades S, Troy DJ (1997) Effects of fat level, oat fibre and carrageenan on frankfurters formulated with 5, 12 and 20% fat. Meat Sci 45: 273-281

Keeton JT (1983) Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. J Food Sci 48:878–881

Koniecko ES (1979) Handbook for Meat Chemist. Wayne, NJ: Avery Publishing Group Inc Pp. 53–55

Maier K, Gormley TR, Connolly PL, Auty M (1997) Assessment of under-utilised fish species. Farm and Food1: 30-34

Raja WH, Kumar S, Bhat SF, Kumar P (2014) Effect of ambient storage on the quality characteristics of aerobically packaged fish curls incorporated with different flours. Springer Plus 3:106

Ramirez JA, Angel AD, Uresti RM, Velásquez G, Vázquez M (2007) Low salt restructured products from striped mullet (*Mugil cephalus*) using microbial transglutaminase or whey protein concentrate as additives. Food Chem 102: 243– 249

Scott DN, Fletcher GC, Charles JC, Wong RJ (1992) Spoilage changes in deep water fish, smooth oreodory during storage in ice. Int J Food Sci Technol 27(5): 577–588

Shabanpour B, Jamshidi A (2013) Combined effects of light salting and microwave pre-drying on the quality of rainbow trout (*Oncorhynchus mykiss*) fish nuggets. World J Fish Marine Sci 5 (5): 497-504

Silva JL, R Eddari-Lynn, Jahncke M, Hearnsberger JO (1994) Effect of washing water pH on color and lipid oxidation of mince and surimi made from gulf menhaden. Proceedings, 19th Trop. Subtrop. Fish. Technol. Conf., Sea Grant, U. Florida, Gainesville

Snedecor GW, Cochran WG (1989) Statistical Methods, 8th edn. Iowa State University Press, Ames, Iowa

Swan JE, Esguerra CM, Farouk MM (1998) Some physical, chemical and sensory properties of chevon products from three New Zealand goat breeds. Small Rumin Res 28:273–280

Tarladgis BG, Watts BM, Younathan MT, Dugan LR (1960) A distillation method for the quantitative determination of malonaldehyde in rancid foods. J Am Oil Chem Soc 37:44-48

Tokur B, Ozkutuk S, Atici E, Ozyurt G, Ozyurt CE (2006) Chemical and sensory quality changes of fish fingers, made from mirror carp (*Cyprinus carpio* L., 1758), during frozen storage (18 °C) Food Chem 99 : 335–341