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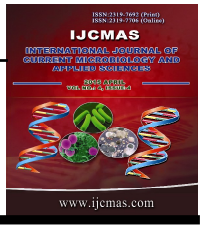

Original Research Article

Chemical composition, minerals content, amino acids bioavailability and sensory properties of meat and fish balls containing fish protein isolate

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Keywords

Fish byproducts, Fish Protein Isolate, Fish and meat balls, Chemical and sensory properties, Minerals content, Amino acids

A B S T R A C T

In this study fish proteins isolated(FPI) by isoelectric solubilization/precipitation processing from unemployed whole small Nile bolti fish and used as meat substitute for preparing ready-to-eat beef and/fish balls. The products were analyzed for chemical composition, minerals, amino acids contents and sensory properties. Also, Essential amino acids index, biological value and protein efficiency ratio were calculated. Incorporation of FPI in meat and fish balls formulae improved protein %, reduced fat and carbohydrate contents of the cooked samples. The essential amino acids index (EAAI) was more than 90% in both meat and/or fish balls. Its value was improved from 94.37 in control sample (100% meat) to 96.78 in the sample containing 25%FPI+75% beef, while a reduction was found in treated samples containing 50%FPI+50% fish. Biological value (B.V.) and Protein efficiency ratio (PER) values assured the recommendation for using FPI with meat in spite of that FPI+ fish is still biologically accepted. The cooked samples containing FPI were of low energetic value, acceptable for consumption with good source for protein and minerals.

Introduction

The fish industry is a major economic source for a number of countries worldwide. The fish processing industry produces more than 60% by-products as waste, which includes head, skin, trimmings, fins, frames, viscera and roes, only 40% fish products are used for human consumption (Dekkers *et al.*, 2011). Commercial filleting of fish such as cod, salmon, tilapia, sea bream, and pollack typically yields approximately 60 - 70%s of by-products and 30% to 40% fillets of the whole fish weight (Torres *et al.*, 2007). Fish industry by-products can account for up to 75% of the catch depending on post harvest or industrial preparation processes, solid wastes generated from seafood factories ranged from 30%-85%s of the weight of the landed fish (Rustad *et al.*,2011). The same authors reviewed that; with a grand total of more than 90 million metric tons wild-caught fish (The Global Education Project, 2011) and a global aquaculture production of more than 70 million metric tons (FAO 2011), the

amount of marine by-products is thus huge. Usually these huge quantities of fish by-product waste create serious pollution and disposal problems in both developed and developing countries; but there is a large potential for making more value-added products from this raw material. The amounts of byproducts in fish vary depending on species, size, season and fishing ground (Falch *et al.,* 2006).

Saritha and Patterson (2014) reported that trash fishes (small fishes) are generally classified in the category of fish that are relatively contained high protein, vitamins and minerals (Nabba *et al.,* 2007). Trash fish is the waste that can be used as a potential resource and so far up only limited trash fishes are being used for traditional products, such as salted fish, fish crackers, fish sauce and fish meal (Renhoran *et al.,* 2011). Rest raw materials from fish processing industry have different properties and are basis for different ingredients and applications. For example Arason, *et al.,* (2009) demonstrated that using fish proteins as ingredients in processing lines for whitefish fillets generally improved the final products. Thus, extension of fish utilization is usually associated with the increase of unemployed stocks such as small fish by-catch and waste of processing which could be utilized economically.

Marine by-products have been reported to be good sources for nutraceuticals and functional food ingredient (Barrow and Shahidi, 2007). Fish by-products contain both valuable lipid and protein fractions as well as other interesting and valuable compounds (Rustad *et al.,*2011). Also, fish wastes are well known to contain highly valued fatty acids including omega-3 fatty acids such as Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA), enzymes (pepsin, trypsin, chymotrypsin), collagen and oil (Swatschek *et al*., 2002; Byun *et al*.,2002; Kim and Mendis,2006).These valuable compounds can be used in the medical and food industries. Therefore, proper utilization of fish wastes can result in a tremendous commercial value. In addition, these fish by-product wastes contain good amount of protein rich material that are usually processed into low market-value products, such as animal feed, fish meal and fertilizer. New industrial processing techniques facilitate to obtain valuable proteins, antioxidants and oils from salmon and rapeseed waste. These extracts can be used in health foods, nutritional supplements and skin care products (SINTEF 2015). Food industry co-streams which could be upgraded to more valuable products than the original ones ending up as animal feed. Scientists developed feasible and gentle methods to make good use of fish filleting residues and rapeseed press cakes (Technical Research Centre of Finland-VTT, 2015).

Proteins possessing functional characteristics and antioxidant activity have immense importance in the food processing industry (Hsu 2010). Fish protein is an essential source of nutrients for many people, especially in developing countries. Upgrade or recovery of edible high-grade protein from fish wastes may assist in solving the protein shortage as reported by Tarky *et al.,* (1973). In view of utilizing these protein rich fish processing by-product wastes, several bio-techniques have been developed to recover the essential nutrients and bioactive compounds that would help to improve human health by protecting from several diseases, as well to solve the pollution and disposal problems (Chalamaiah *et al.,*2012). Tahergorabi *et al.,* (2012) reported that recovered proteins retain functional properties and nutritional value as indicated in several studies.

Fish protein fraction is easily digestible and can be used for the production of hydrolysates, surimi, thermo-stable protein dispersions, different peptides and amino acids, gelatin and collagen as well as protamine (Rustad *et al.,* 2011). Different types of protein fractions can be produced from fish rest raw material e.g. flesh from backbones and cut-offs can be used for the production of fish mince, surimi or products based on surimi (Taylor and Himonides, 2007). Production of fish protein ingredients is growing throughout the world. There are several methods to produce fish protein ingredients such as surimi, minced fish, and fish protein isolate, etc. Fish protein isolate (FPI) is a protein concentrate prepared from fish muscle without retaining the original shape of the fish muscle. Generally it is not consumed directly, but used as raw material in the food industry. FPI can be used as an ingredient for production of value added and ready-to-eat products based on minced fish or surimi and still a good source of protein for the production of ready to eat fish products (Shaviklo 2006; Garba and Kaur, 2014; Shaviklo *et al.,* 2010). FPI is a type of concentrated fish protein that has been purified to have a protein content of at least 90% of the dry material. However, the term FPI is most often used for fish muscle proteins that have been produced by the pH shift process, which is seen as a more efficient process for raw materials such as whole fish or fish rest raw materials (Kristinsson and Hultin, 2003 ; Kristinsson and Liang, 2006; Thorkelsson *et al.,* 2008). Tahergorabi *et al.,* (2012) in their study demonstrated a feasibility to develop functional food products made of muscle protein isolate recovered with isoelectric solubilization /precipitation from whole gutted fish (bone-in, skin and scale-on).

According to FAO (2010) Egypt is by far the main Tilapia producer from fresh and brackish waters of capture fisheries as well as from aquaculture in the MENA region. Also, according to the Egyptian General Authority for Fish Resources Development, about 477.458 tones of Tilapia fish consisting of 55.6% of the total Egyptian production from fish culture in 2008. Worthy to mention that Egyptian production represents 12% of the world farmed Tilapia (2.121.009 tones) (FAO 2008). This was consistent with the FAO fisheries statistics, total Middle East and North Africa (MENA) region production from fresh fishers that showed a steady rise along the period from 2004-2008. Egypt produces over 92% of total Tilapia production in Middle East and North Africa (MENA) region. *Tilapia nilotica* is of high economic value for aquaculture because it can be raised to uniform and marketable sizes within a short period of time (khalil 2012).

Thus, considering the increasing of consumer demand for ready to eat food products with high nutritional and health values, in addition of economical and environmental benefits of using fish byproducts in food industry, the main objective of this study was to develop a ready to eat meat and/fish product containing fish protein isolate and so it was aimed to: i) Prepare Fish Protein Isolate(FPI) from unemployed stocks of whole small fish by-catch; ii) Use the prepared FPI as a partial meat substitute for preparing beef and/ fish balls, and iii) Investigate the effects of using FPI on chemical composition, minerals content and sensory properties of the prepared products as well as amino acids content. Also, essential amino acids index, biological value and protein efficiency ratio were calculated to estimate the parameters related to the biological values of products under investigation.

Materials and Methods

Materials

Fresh Nile bolti fish (*Tilabia nilotica*), small and large size, were obtained from local fish market and transported in ice-box to the laboratory. Upon arrival, the fish were vacuum-packed in polyethylene bags and stored at (-20±2°C) until used. The proximate composition, pH and minerals content of small fish were determined.

Fresh deboned beef meat from the hind quarter of slaughter males of two years old was obtained from local market and stored at 4±1ºC for approximately 4 hours before use.

Food ingredients (Table1) were bought from the local market and were used in the suggested meat and/or fish balls formulations. All of chemicals used were of analytical grade.

Methods

Preparation of Fish protein isolate

The whole small bolti fish (bone-in, skin- and scale-on) were the input material for the isoelectric solubilization /precipitation (ISP) processing to recover muscle protein isolate. Protein was isolated from the minced whole small Nile bolti fish sample using an ISP method according to *Tahergorabi et al.,* (2012). Briefly, fish was blended in a1:6 w/v fish: deionized distilled water (ddH_2O) mixture. The fish ddH_2O mixture was homogenized at a temperature of 4ºC and the pH adjusted to 11.5 using NaOH solution. The fish homogenate was centrifuged at 10,000 *g* for 10 min and the supernatant collected. The pH of the supernatant was adjusted to 5.5 using HCl solution then centrifuged at 10,000 *g* for another 10 min. The resultant FPI was collected and stored at -20ºC

Preparation of Meat and Fish ball samples

The beef meat and large bolti fish flesh were minced using house meat mincer then mixed with the other ingredients in (Table1) without adding fish protein isolate (FPI) and used for preparation of meat or fish balls as control samples (1&2). The minced beef, minced fish and FPI along with the other ingredients were mixed in the food homogenizer and used for preparation of treated meat and fish ball samples containing FPI (samples 3&4). The prepared meat and/or fish ball samples were as follows:

- Control samples:
Sample [1] Meat ball (100% beef meat),
Sample [2] Fish ball (100% fish flesh).

- Treated samples:
Sample [3] FPI + Meat ball (25% FPI + 75% beef meat).
Sample [4] FPI + Fish ball (50% FPI + 50 % fish flesh).

Forming of all meat and fish ball samples was done manually under hygienic conditions (each piece about 40 grams). Thermal processing for setting was a combination of boiling in hot water (98±1°C) for 7 minutes followed by deep frying at (180±2°C) for 1 min (precooking). Meat and fish ball samples were blast frozen at-18°C after setting, cooling and packaging in polyethylene (PE) bag**.**

Analytical methods

Proximate composition

The moisture, total protein, fat and ash contents were determined in accordance with standard AOAC methods (AOAC 1995). Total carbohydrates were calculated by difference. Energetic value (cal. /100gm)

of the cooked samples was calculated using the following equation:

Energetic value = [(% of carbohydrate x 4.1) + (% of protein x 4.1) + (% of fat x 9.1)]

All results were recorded as the mean value of 3 replicates.

pH value

Measurement of pH of all the tested samples was carried out on 10g of sample homogenized in distilled water (1/10 sample/water). The pH values were measured using a standardized electrode attached to a digital pH meter (HAANA, HI902 meter Germany). All samples were measured at room temperature. The pH value was the average of three readings.

Cooking loss

Following draining of meat or fish balls after boiling/frying, the samples were put on a layer of paper towels to remove the excess moisture and oil then left to room temperature. Five meat and/or fish balls were selected randomly and weight directly. The cooking loss was calculated according to Alleson-Carbonell *et al.,* (2005) as follows:

Cooking loss %= $[(P_1 - P_2)/P_1] \times 100$, where:

P_1= Initial weight of meat and/or fish balls samples (g) and

P_2= weight of meat and/or fish balls after boiling and frying (g).

Cooking yield

Cooking yield was determined according to Alleson-Carbonell *et al.,* (2005) by dividing cooked product weight by the raw uncooked weight and multiplying by 100.

Sensory evaluation

Frozen meat and/or fish ball samples with or without FPI were removed from the freezer and put into a refrigerator for thawing overnight. Fresh and thawed samples were cooked into hot-air oven at 270°C for 3 minutes.

The samples were coded with random numbers and served to be evaluated in terms of general acceptability, texture, flavor by 10 trained panelists using 0-9 point hedonic scale descriptive analysis. According to the following scoring, (7-9) indicated "high quality", (4-6) indicated "moderate quality" and (1-3) indicated the limit of "unacceptability" according to Weber *et al.,* (2008).

Amino acids

A full amino acids profile analysis was conducted according to AOAC method (AOAC 1995). The meat and/or fish ball samples with or without FPI were hydrolyzed with 6 N HCl for 24 h.

Amino acids were quantified using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter Inc., Fullerton, Calif., U.S.A.) employing sodium citrate buffers as step gradients with the cation exchange post-column ninhydrin derivatization method. The data were reported as milligrams of amino acid per 1 g of sample.

Essential amino acid index (EAAI)

The (EAAI) was calculated according to Oser (1959) as the geometric mean of the ratio of essential amino acids and the food protein relative to their respective amounts in whole egg protein expressed as percentages.

Biological value (B.V.)

The (B.V.) was assayed according to the following equation which recommended by Oser (1959)**.**

B.V. = 1.09(EAAI) – 11.73

Protein efficiency ratio (PER)

The protein efficiency ratios of the tested samples were based on their amino acids contents according to the recommendations of Alsmeyer(1974). However, the following equations were used:

PER= - 0.684+0.456(Leucine) - 0.047(Proline)

PER= - 0.468+0.454(Leucine)– 0.105(Tyrosine)

PER=-1.816 +0.435(Methionine) +0.78(Leucine+0.211(Histidine)- 0.944(Tyrosine)

Minerals

All glassware was washed overnight in a solution of 10% HCl in deionized distilled water (ddH_2O, v/v) prior to use. Ashed samples were dissolved in 2 mL of 70% nitric acid. The acidified samples were neutralized in 5 mL of ddH_2O and filtered through Whatman No.1 paper. Samples were diluted to volume with ddH_2O in a 50-mL volumetric flask (Chen *et al.,* 2007). Minerals content were determined using inductively coupled plasma optical emission spectrometry (model P400; Perkin Elmer, Shelton, Conn., U.S.A.)

Statistical analysis

The obtained data were presented as means and standard deviation (mean±SD) and subjected to analysis of variance (ANOVA) according to PC STAT, Version I A Copyright 1985, the University of Georgia (PCSTAT, 1985)**.**

Result and Discussion

Chemical composition

Fish byproducts and underutilized fish species that usually are not used directly for human food can be utilized in pH shift process (Hultin *et al.,* 2005). On the other hand, whole fish with skin bones and fatty fish can be used in this process technology because proteins are selectively separated and isolated from undesirable materials (Krisitinsson *et al.,* 2006)

The small fish that are not utilized for human consumption is discarded and has little economic value because of their small size, limited appeal to consumers and fall into the by-product category (Jeyasanta *et al.,* 2013)**.** Also, It has been cleared by the same authors that the utilization of low valued by-catches for human consumption is mainly done in the form of mince based products e.g. fish sausage, cakes, patties, balls, pastes, fingers, surimi, etc, or fish protein concentrate with or without bone.

In the present work Nile small bolti fish were the raw material that used for preparing fish protein isolate (FPI) in order to be as a partial meat substitute in processing meat (beef) and/or fish balls as suggested ready-to-eat products**.** Proximate composition of small Nile bolti fish (*Tilapia nilotica*) in addition to the prepared FPI was concerned to shed the light on the chemical composition of the investigated samples. Proximate analyses like moisture, protein, fat and ash content were analyzed to assess the nutritional quality of tilapia fish (Dhanapal *et al.,* 2012).

Data presented in Table (2) showed high moisture content in both whole small fish (76.33%) and its flesh reached (78.21%). El-Serafy *et al.,* (2005) reported that the water content in Nile Tilapia fish muscles was higher in immature than mature fish. The present results agreed Galhom (2002) who indicated that, moisture content of fish from Egyptian waters ranged between 70.00 and 79.00%. Also, Abd-Alla (1994) found a range between 80.50 and 48.00% for moisture content of fish muscles from various fish cultures. Thus, water being the most important constituent contributing maximum value to the chemical composition in fish tissues and can show high degree of variation.

The ash and fat contents of the studied whole small bolti fish showed higher %s than their flesh. This was expected due to the presence of bone-in, skin- and scale-on in whole fish. El-Zaeem *et al.,* (2012) reported in their study that it is very difficult to ascertain the optimum level of fat in a carcass also they felt generally, that fat percentage of 16 to 18 is too high for fillet of Nile Tilapia fish and the increase in fat depots increases waste during processing. It was noticed that pH of the investigated bolti fish and their flesh had nearly the same values (Table 2).

Isoelectric solubilization/precipitation (ISP) allows efficient recovery of fish protein isolate (FPI) that could be used in functional foods (Tahergorabi *et al.,* 2012).

In the present study, whole small bolti fish include (bones, skin, scales, fins and viscera in addition to flesh) were used for producing fish protein isolate (FPI) applying ISP processing. It was observed from the data in (Table 2) that FPI revealed the absence of carbohydrates content and this can agree with the results of Shaviklo *et al.,* (2012). The increase of FPI protein level was noticed to be associated with decrease in fat level; this can be attributed to removal of fat through the processing of FPI. On the other hand, FPI ash content was noticed to be reduced from 20.05 to 13.80% (on dry weight) compared to whole small bolti fish. ISP processing allows selective, pH-induced water solubility of muscle proteins with concurrent separation of lipids and removal of materials not intended for human consumption such as bones, scales, skin, etc. (Gehring *et al.,* 2011). Chen *et al.,* (2007) noticed that the ISP of trout processing by-products yielded a protein with lower ash content than the fillets, and the vast majority of ash was retained with the recovered.

Minerals

The mineral elements which the body required are frequently classified as either macro-or micro-nutrients, depending on the amount of each that is need in diet. Calcium, phosphorus, potassium, sulfur, chlorine, sodium and magnesium are considered macronutrient elements. Iron, iodine, fluorine, zinc, copper, chromium, selenium, cobalt, manganese, molybdenum, vanadium, tin, silicon and nickel are often classified as micro-nutrient or trace element (Ibrahim1986). Fish muscle contains minerals, vitamins and other nutritional compounds which are necessary in a diet (Larsen *et al*., 2007)**.**

Some minerals of the investigated small bolti fish and FPI samples were given in Table 3. The obtained results showed that contents of Ca, P, Na, Mg, Fe, Mn, Zn, varied greatly. The different individual minerals were found with higher concentration in whole small fish sample. This may be related to the higher ash content of whole small fish sample (Table 2). Furthermore, the source of high mineral

content was mainly from bones of the fish, which were otherwise discarded (Jeyasanta *et al.,* 2013). The presently studied small fish were characterized by highest level of Ca followed by Na then P. The balance of mineral ions is important since the amount and ratio of Ca / P are of great significant for normal muscular activity as reported by Ibrahim (1986).

Generally, FPI results given in Table 3 pointed out to the reduction of minerals. Ca, P and Mg contents in the recovered protein (FPI) were greatly reduced when compared to the whole small bolti fish. These minerals may be removed from the protein recovered from the whole small bolti fish due to the centrifugation step following protein solubilization. This was also confirmed by the low ash content in the recovered protein as shown in Table 2. This agreed with Chen, *et al*., (2007) who reported that the major minerals Ca, P, and Mg are involved in bone health; thus the insolubles recovered during ISP may provide a good mineral source for the prevention of bone loss. In addition, ISP removed minerals from the recovered protein without removal of the bones, skin, scales, fins, and so on prior to processing. The minerals were concentrated in the insoluble recovered with the basic treatment and could potentially be used as a supplementing mineral for the human and/or animal diet. Thus the above-mentioned results can support the view that the composition of the raw materials (fish and FPI) was markedly rich in protein and numerous of the required minerals needed for human health.

Proximate composition of meat and fish balls samples

Several attempts have been made for utilization of the protein rich fish processing by-product wastes and underutilized fish proteins for the production of commercially valuable food ingredients (Chalamaiah *et al.,* 2012). Ready-to-eat fish products were processed by mixing minced or surmi fish with different ingredients. FPI can be used as a fish protein ingredient or even replacer of whole / or a part of mince in the formula (Shaviklo 2008).

In the present study, meat ball based on 25% FPI and 75% beef meat as well as fish ball containing 50% FPI and 50% bolti fish flesh (large fish mince) were suggested as ready-to-eat fish and/or meat products; meanwhile meat and fish balls based on 100% beef meat or fish flesh without adding FPI were used as control. All meat and fish balls under investigation were prepared and analyzed.

Data of proximate composition of meat and fish balls prepared with adding FPI was depicted in Table 4. The data indicated that no remarkable changes between moisture contents of the fresh control (1&2) and treated samples with adding FPI (3&4).Concerning protein %, it could be noticed that the addition of FPI improved the crude protein for both treated samples (3&4) whereas their protein contents reached 52.20 and 61.73 %s respectively on dry weight basis (Table 4). Regarding the ash content of the studied fresh meat and/or fish ball samples, it was found that this content reached 9.08 % for the meat ball with FPI (sample 3) and 11.76 % for the fish ball with FPI(sample 4) compared to 6.69 and 9.08 % for the control without FPI (samples 1&2) respectively. Incorporation of FPI in meat and fish balls formulae reduced the fat and carbohydrate contents of the samples and so the energetic value of meat and fish ball samples could be reduced

With respect to the cooked samples it was found that the treated samples 3 & 4

contained FPI had higher amounts of moisture than the control samples 1 & 2 this may be due to the thermal denaturation of proteins. Alipour *et al.,* (2010) showed that the heating treatments denature the proteins of sturgeon fillets. Also, this increment may be due to sodium alginate which capture moisture and consequently improved the water holding capacity of these samples. Worthy to know that moisture loss is a critical factor that affects the quality and acceptability of cooked meat products. Since the meat and/or fish balls as read-to-eat are usually subjected to precooking, freezing and later reheating, it is desirable to have tenderness and moistness retained through a suitable formulation strategy plan.

In the cooked samples containing FPI (Table 4) a reduction was noticed in fat and carbohydrates %s in the treated samples 3&4 compared to the control cooked samples1&2. Therefore, Energetic value of the cooked samples was calculated (Materials and method part).The calculated energetic values of the cooked control samples (1&2) without FIP were 615.196 and 591.730 cal./100gm, and these values decreased to 570.301 and 511.739 cal./100gm respectively in the treated cooked samples (3&4) containing FPI. Hence samples (3 & 4) with FPI were of low energetic values; so they can be recommended as functional food with low energy values (as regiments food).

Regarding pH values in the same Table it could be observed that addition of FPI to fresh control meat and fish ball samples (1&2) raised the pH of fresh treated meat and fish samples (3&4) to reach 6.13 and 6.60 respectively. Dhanapal *et al.,* (2012) indicated that the increase in pH of the samples may be due to breakage of hydrogen bond and electrostatic interactions, also the highest pH values of the cooked samples might be related to protein denaturation which was caused by heating as one of the factors that may raise the pH value.

Minerals of meat and fish balls samples

With respect to mineral contents of the studied meat and/or fish balls, data in Table 5 indicated generally that the fresh control fish balls (sample 2) mostly had higher contents (except Mn) than control meat balls (sample1).

Addition of FPI to the fresh treated meat balls (sample 3) enhanced Ca, Na, Mg, Fe, Mn and Zn contents (except of P content). Regarding to adding FPI to fish balls (sample 4) increased Ca, Na and Mn levels; while a reduction of P, Mg, Fe and Zn levels was occurred. This can be due to the different minerals composition of fish flesh and the FPI. It was also noticed that the addition of FPI may reduce P content of fresh treated meat and/or fish balls samples (3&4).

Data in the same Table showed generally that the levels of minerals content in the cooked fish ball samples were still higher than that of the cooked meat ball. According to cooked control meat ball sample without FPI (sample 1) it was noticed a reduction in P, Na, and Fe, while Mg and Na %s were decreased in the treated fish ball sample without FIP (sample 2) relative to the fresh control samples1&2 respectively.

Worthy to note that addition of FPI improved most of mineral levels of the treated meat ball sample 3; but lower levels of Mg, Fe and Zn in the cooked treated meat ball sample 4 were observed. However, the studied cooked fish/or meat ball still considered as a good source for minerals needed for human.

Amino acids bioavailability

Nutrient bioavailability is a complex subject when applied to protein components. In fact, it is not quite write to speak of the bioavailability of the protein without considering the bioavailability of the individual amino acids which form up the protein structure. On the other hand, one may chemically analyze the amino acids of a protein, but this analysis is the best viewed as an estimate of the potential nutritional value of the protein, through calculating essential amino acids index (EAAI), biological values and protein efficiency ratio. Fish is an excellent protein source with high nutritive value due to a favorable essential amino acid composition (Larsen *et al*., 2007).

In the present study amino acids content of the meat and/or fish balls samples with adding FPI given in Table 6 proved the presence of the normal detectable amino acids of which glutamic acid was 16.01 g/16gN and aspartic acid came in second order with 7.63 g/16gN in meat balls containing 100% beef (control sample1) also the fish balls containing 100% fish (control sample 2) showed same order with16.70 and 8.48 g/16g N for glutamic and aspartic acids respectivly. Glutamic acid is a free amino acid found in skeletal muscles at around 60% rate in human body (Christina *et al.*, 1999). With respect to amino acid values of leucine (7.23g/16gN) and lysine (7.95g/16gN) of meat balls(control sample1) and fish balls (control sample 2)came in the third order. Iso-leucine level was nearly similar in both control samples 1&2. There are some slight differences between threonine, methionine, tryptophan and serine amino acids. It was indicated also that glycine (6.81g/16gN)and proline (4.40 g/16gN) in fish ball sample 2 were higher than in meat ball sample1 (5.18 for the former and 2.81g/16gN for the latter respectively) whereas histidine value was lower(1.80g/16gN) in fish ball than meat ball sample (3.00 g/16gN). Glycine is an important amino acid as it is one of the main components of human connective tissue (Mat Jais, *et al.*, 1994**).**

Data in Table 6 showed the amino acids content in the prepared meat and/or fish balls samples(3&4) containing FPI as partial meat substitute, in relative to control samples(1&2) of 100% beef and 100% fish balls without adding FPI. These data indicated the occurrence of glutamic and aspartic acids in the same order of their presence in the meat and/or fish balls samples (1&2). Variations were noticed within small scale limit between amino acid values of meat and fish balls.

EAAI, B.V., and PER are considered as important parameters for quality comparison based on the estimation of protein quality The EAAI was more than 90% in the meat and /or fish balls. Its value was improved from 94.37 in sample1 (100% meat) to 96.78 in the sample 3 containing 25% FPI +75% beef. On the contrary, a noticeable reduction was found in sample 4 (50% FPI + 50% fish). This may be due to the proteolytic enzymes media in the latter sample and subsequently tended to hydrolyze a portion of the protein to free amino acid which could be affected hardly through acid hydrolysis. Such trend of the enzyme in the former sample (heterogeneous) did not actually occurred in the latter sample due to the long adaptation of the proteolytic enzyme towards its substrate. The B.V. and PER values (Table 6) proved the recommendation of using FPI with meat in spite of that FPI + fish is still biologically accepted.

Table.1 Formulae for preparation of meat and/or fish balls samples with Fish protein isolate (FPI)

Ingredients (%)	Control samples		Treated samples	
	Sample 1 Meat(beef)	Sample 2 Fish flesh	Sample 3 25% FPI + 75%Meat	Sample 4 50% FPI+50%Fish
Beef mince	70.0	-	52.5	–
Large bolti fish flesh mince	–	70.0	–	35
Fish protein isolate (FPI)	–	–	17.5	35
Fresh onion	2.0	2.0	2.0	2.0
Fresh garlic	2.0	2.0	2.0	2.0
Sodium chloride	2.0	2.0	2.0	2.0
Sunflower oil	9.5	9.5	9.5	9.5
Wheat flour	3.0	3.0	3.0	3.0
Bread crumbs	8.0	8.0	8.0	8.0
Spices mix.*	3.0	3.0	3.0	3.0
Sodium alginate	0.5	0.5	0.5	0.5
Total	100	100	100	100

* Spices mix. =Powdered mixture prepared from equal amounts of cummin, cordiander, cloves, nut meg, black pepper, red pepper, cubeb, cardamon and mastic.

Table.2 Proximate composition* of small bolti fish and Fish protein isolate (FPI)

Samples	Moisture%	Protein%	Fat%	Ash%	Carbohydrate%	pH
			Small fish			
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Whole fish	76.33±0.000^{d}	63.57±0.031^{e}	16.05±017^{b}	20.05±0.023^{a}	1.32±0.015^{b}	6.43±0.009^{a}
Fish flesh	78.21±0.031^{b}	84.83±0.031^{a}	7.00±0.027^{d}	7.15±0.011^{d}	1.02±0.015^{c}	6.30±0.009^{a}
			Fish protein isolate (FPI)			
FPI	78.65±0.031^{a}	83.90±0.000^{b}	2..30±0.009^{e}	13.82±0.047^{c}	0.00±0.000^{e}	5.70±0.100^{b}

*Calculated on dry weight basis Letters a-e to show significant differences (P< 0.05) between the same column.

Table.3 Some Minerals content*(mg/100gm) of Bolti fish and Fish protein isolate (FPI)

Sample	Ca	P	Na	Mg	Fe	Mn	Zn
			Small fish				
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Whole fish	2385.97±0.100^{a}	1046.46±0.106^{b}	1411.75±0.100^{a}	396.80±0.102^{a}	41.27±0.100^{b}	12.39±0..999^{a}	30.95±0.999^{b}
Fish flesh	784.48±0.100^{c}	1322.23±0.100^{a}	442.67±0.102^{c}	362.11±0.11^{b}	43.54±0.100^{a}	0.37±0.010^{c}	54.39±0.999^{a}
			Fish protein isolate (FPI)				
FPI	1583.99±0.123^{b}	648.23±0.968^{c}	709.47±0.968^{b}	244.91±0.101^{c}	21.30±0.999^{c}	9..31±0.100^{b}	15.98±0.100^{c}

*Calculated on dry weight basis Letters a-e to show significant differences (P< 0.05) between the same column.

Table.4 Proximate composition* of meat and fish balls prepared with adding Fish protein isolate (FPI)

Samples	Moisture%	Protein%	Fat%	Ash%	Carbohydrate%	pH
			Fresh samples			
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control samples						
Sample1	61.36±0.022^{c}	46.90±0.027^{d}	43.68±0.035^{a}	6.69±0.015^{c}	5.62±0.009^{a}	5.95±0.957^{d}
Sample 2	68.45±0.000^{a}	47.01±.022^{c}	41.40±0.035^{b}	9.08±0.015^{b}	5.51±0.025^{b}	6.32±0.025^{b}
Treated samples						
Sample 3	61.26±0.022^{d}	52.20±0.016^{b}	37.08±0.016^{c}	9.08±0.015^{b}	4.63±0.010^{c}	6.13±0.020^{c}
Sample 4	68.08±0.031^{b}	61.73±0.022^{a}	26.25±0.022^{d}	11.76±0.015^{a}	3.24±0.015^{d}	6.60±0.035^{a}
			Cooked samples			
Control samples						
Sample 1	59.14±0.022^{d}	44.95±0.022^{d}	44.45±0.000^{a}	7.12±0.000^{d}	6.44±0.020^{a}	6.10±0.009^{d}
Sample 2	67.10±0.031^{b}	46.95±0.027^{c}	40.97±0.022^{b}	9.43±0.019^{b}	5.63±0.015^{b}	6.55±0.019^{b}
Treated samples						
Sample 3	63.33±0.022^{c}	51.65±0.0271^{b}	37.30±0.022^{c}	9.37±0.025^{c}	4.66±0.015^{d}	6.30±0.009^{c}
Sample 4	71.83±0.000^{a}	60.46±0.000^{a}	26.67±0.156^{d}	10.69±0.016^{a}	5.16±0.020^{c}	6.85±0.020^{a}

*Calculated on dry weight basis

Control samples: Sample 1= Meat (100%beef), Sample 2= Fish (100% fish).

Treated samples: Sample 3= FPI+ Meat (25% FPI + 75% beef). Sample 4= FPI+ Fish (50% FPI + 50 % fish flesh).

Letters a-e to show significant differences (P< 0.05) between the same column.

Table.5 Some Minerals content*(mg/100gm) of the meat and/or fish balls with Fish protein isolate (FPI)

	Ca	P	Na	Mg	Fe	Mn	Zn
			Fresh samples				
Samples	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control samples							
Sample 1	55.40±0.965^{d}	996.40±0.106^{c}	2602.00±0.100^{d}	13.06±0.101^{d}	14.68±0.035^{c}	15.56±0.016^{b}	15.65±0.015^{d}
Sample 2	918.01±0.100^{b}	1123.95±0.112^{a}	3593.83±0.141^{b}	416.20±0.960^{a}	45.12±0.015^{a}	0.26±0.010^{d}	57.41±0.022^{a}
Treated samples							
Sample 3	271.35±0.988^{c}	355.93±0.960^{d}	2804..33±0.100^{c}	118.40±0.989^{c}	16.68±0.025^{b}	15.88±0.011^{a}	19.26±0.021^{c}
Sample 4	1187.31±0.866^{a}	1097.11±0.935^{b}	3621..22±0.000^{a}	402.19±0.992^{b}	3.43±0.009^{d}	3.44±0.015^{c}	48.30±0.016^{b}
			Cooked samples				
Control samples							
Sample 1	61.46±0.999^{d}	227.14±0.100^{d}	2482..33±0.100^{d}	80.33±.999^{d}	11.24±0.009^{d}	17.51±0.100^{b}	18.18±0.999^{d}
Sample 2	955.33±0.106^{b}	1339.83±0.118^{b}	3352..38±0.014^{b}	393.99±0.100^{a}	48.33±0.100^{a}	0.272±0.009^{d}	62.06±0.100^{a}
Treated samples							
Sample 3	285.67±0.100^{c}	621.29±0.103^{c}	2949.00±0.100^{a}	112.20±0.1000^{c}	13.61±0.100^{c}	20.24±0.100^{a}	21.74±0.100^{c}
Sample 4	1109.17±0.935^{a}	1494.18±0.100^{a}	3960.10±0.000^{a}	330.05±0.101^{b}	33.32±0.100^{b}	3.12±0.999^{c}	48.92±0.999^{b}

*Calculated on dry weight basis

Control samples: Sample 1= Meat (100%beef), Sample 2= Fish (100% fish).

Treated samples: Sample 3= FPI+ Meat (25% FPI + 75% beef). Sample 4= FPI + Fish (50% FPI + 50 %fish flesh).

Letters a-e to show significant differences (P< 0.05) between the same column.

Table.6 Amino acids content (g/16 g N) and responsible evaluating parameters of meat and/or fish balls with adding Fish Protein Isolate (FPI)

Amino acids	Meat ball containing:		Fish ball containing:	
	100%meat(beef) Control sample 1	25%FPI+75%meat Treated sample 3	100%fish Control sample 2	50%FPI+50%fish Treated sample 4
Essential Amino Acids				
Lysine	6.73	7.67	7.95	7.77
Iso-leucine	4.00	3.93	3.94	4.02
Leucine	7.23	7.03	6.89	8.47
Methionine	1.38	1.60	1.62	1.43
Phenylalanine	3.65	3.33	3.34	3.26
Threonine	3.70	3.93	3.86	3.76
Tryptophan	1.13	1.07	1.25	1.15
Valine	4.52	4.50	4.60	4.46
Histidine	3.00	2.80	1.80	1.70
Non-Essential Amino Acids				
Arginine	5.44	4.76	5.30	5.10
Aspartic	7.63	7.94	8.48	8.00
Serine	3.50	3.73	3.71	3.51
Glutamic	16.01	15.9	16.70	15.23
Proline	2.81	3.73	4.40	3.51
Glycine	5.18	4.50	6.81	5.58
Alanine	5.53	6.44	5.94	5.38
Cystine	-	-	-	-
Tyrosine	2.70	2.41	2.12	2.69
Evaluating Parameters				
EAAI	94.37	96.78	98.88	93.61
B.V.	91.04	93.68	95.97	90.21
PER*	2.57	2.77	2.53	1.97

*Avarage of Alsmeyer's equation.

Table.7 Sensory evaluation, cooking loss and cooking yield %s of the tested cooked meat and/or fish balls samples

Mean scores of sensory evaluation of the tested meat and/or fish balls samples				
Samples	**Sensory parameters:**			
	General acceptability Mean±SD	Odor Mean±SD	Texture Mean±SD	Flavor Mean±SD
Control samples				
Sample 1	6.30±0.010[a]	4.10±0.009[d]	6.15±0.010[d]	6.38±0.016[a]
Sample 2	5.70±0.015[b]	4.8 0±0.015[b]	7.00±0.015[c]	5.90±0.025[b]
Treated samples				
Sample 3	5.01±0.025[c]	4. 65±0.025[c]	7.64±0.020[a]	5.53±0.015[d]
Sample 4	4.65±0.015[d]	4.90±0.015[a]	7.40±0.090[b]	5.60±0.035[c]

Cooking Loss and Cooking Yield %s of the tested meat and/or fish balls samples		
	Cooking Loss%	**Cooking Yield%**
Control samples		
Sample 1	3.23±0.019[b]	97.80±0.031[c]
Sample 2	3.51±0.025[a]	97.48±0.000[d]
Treated samples		
Sample 3	1.55±0.020[d]	99.42±0.031[a]
Sample 4	2.49±0.019[c]	98.50±0.031[b]

Control samples: Sample 1= Meat (100% beef), Sample 2= Fish (100% fish).
Treated samples: Sample 3= FPI+ Meat (25% FPI + 75% beef), Sample 4= (50% FPI+ 50% fish flesh)
Letters a-e to show significant differences (P< 0.05) between the same column.

Sensory evaluation, cooking loss and cooking yield %s

The sensory qualities of the investigated meat and/or fish balls with or without FPI were evaluated in terms of general acceptability, odor, texture and flavor according to data depicted in Table 7.

Data revealed that control sample 1 (100% meat) had highest scores for general acceptability and flavor. Fish flavor was noticed in control sample 2 (100% fish) and treated samples 3&4 containing FPI. Worth mentioning to observe that although the texture of samples 2,3 and 4 was of high quality scored; grainy texture was detected in samples 3&4 containing FPI and control sample 2. This may be due to the temperature and ingredients used in preparing and cooking of the products. Odor of added ingredients (e.g. spices, garlic and onion) was detected in all samples. Control sample 1 had the lowest score whereas fish balls with 50% FPI (sample 4) was of the highest score followed by control sample 2 then sample 3. However, it can be noticed that nearly there is no considerable differences between control sample 2 (100% fish) and samples containing FPI. The overall acceptability of all samples produced a moderate score.

Cooking loss and cooking yield %s

Cooking loss and cooking yield %s were calculated for the cooked meat and/or fish ball samples (Table 7).It is clear that the highest cooking yield was for the treated sample 3 (99.42%) followed by sample 4 (98.50%) then both the control meat and fish samples (1&2) realized 97.80 and %s 97.48 respectively. Cooking loss % was noticed to be reduced in treated samples contained FPI 3&4. Thus it means that the presence of FPI in samples 3and4 proved higher cooking yield and low cooking loss %s compared to the control samples 1&2.

Utilization of fish byproducts and underutilized fish species is one of the most important challenges in fish industry. Small Fish by-catch and discards fall into the byproduct category. Significant value could be added if protein were recovered from the fish byproducts for use in human food products. Therefore, development of an efficient protein recovery technology is highly desirable. This study demonstrated a feasibility to develop meat and/fish balls as ready-to-eat products using protein isolate recovered with isoelectric solubilization/ precipitation from whole small bolti fish. The suggested meat and/fish balls with FPI were nutritionally enhanced whereas protein improved, fat decreased, with low energetic value and can considered as good source for minerals needed for human health.

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