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Original paper

Effect of Partially Substitution of Rice bran and Soybean Protein Concentrates on Chemical, Nutritional and Organoleptic Properties of Kapreeta tuna-like fish (*Scombromorous* Spp.) Surimi

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Abstract

The objective of this research is to study the possibility of utilizing kapreeta fish (like tuna) that has low nutritional value because of its bloody dark flesh and presence of many blood vessels and susceptibility to rapid spoilage, therefore. Kapreeta fish was used to prepare a high nutritional value of Surimi blends prepared from fish flesh supplemented with 15% level of soybean and rice bran protein concentrates to suggest its protein as a new protein source. Various compositional and functional properties were assessed to determine the effect of this substitution treatment on the final product characteristics. The obtained results revealed that: rice bran protein concentrate is considered a good source of protein (72.90% on dry matter), more than 48.10 and 35.60% for soybean protein concentrate, whereas ash content of soybean protein (5.83%) are higher than that its value of rice bran protein concentrate (4.47%). The two protein sources showed to be rich in essential amino acids and met human requirements for all the essential amino acids since they have higher values than that recommended by FAO/WHO (1991) pattern for children of 10-12 years.

Keywords Rice bran protein concentrates, soybean protein concentrate, Kapreeta tuna-like fish, Surimi.

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Introduction

Agriculture byproducts like rice bran store solar energy in their chemical connections, making them a renewable resource. According to **FAOSTAT (2012),** the world's rice output in 2010 was 696,324,394 metric tons (t), whereas Egypt's production was 5,724,103 metric tons (t) each year **(A.S.G.A., 2013)**. When paddy is milled, it generates 70% rice (endosperm) as the main product, along with 20% husk, 8% bran and 2% germ. Approximately 90 percent of the world's rice bran is thrown away or used as a low-cost animal feed component, with the remainder being used to extract rice bran oil **(Schramm et al., 2007; Zullaikah et al., 2009 and Gul et al, 2015).** During the milling process to create white rice, a combination of rice bran (brown layer) and germ is produced as a byproduct. Die Rice Bran Composition varies depending on the Rice Type and Variety as well as the Rice Processing Methods and Climate Conditions **(Saikia and Deka, 2011)**. As a by-product of the rice milling process, defatted rice bran is seen as a waste product. Bioactive substances such as phenolics give it a significant antioxidative effect **(Devi and Arumughan, 2007; Mariod et al, 2010)**. There are vital nutrients such as high-quality proteins with distinct nutritional value **(Gul et al, 2015)**. As a result of protein-energy malnutrition, which is a worldwide concern, especially in poorer nations, proteins are considered the most important macronutrient for humans. Recent years have seen a rise in interest regarding the possible use of protein from under-utilized biomass sources, such as oil meal and legumes **(Otten et al., 2006)**. Because of the limited use of chemical analysis, which is cheap and rapid, but does not always match in vivo results, the assessment of protein quality has depended on indirect methods such as chemical analysis. Nutritionists use protein quality as a measure of bioavailability to determine how well dietary proteins and diets can meet metabolic needs for amino acids and nitrogen, respectively **(Gilani, 2012)**. In the food business, rice bran protein concentrate (RBPC) has gained attention for its unique nutritional value and nutraceutical characteristics. Even though rice bran protein has the potential to be a novel kind of protein, very little research has been done on its nutritional value **(Han et al., 2015)**.

Soy protein is derived from soybeans. It is produced from dehulled and defatted soybean meal. Only soybean protein includes all of the necessary amino acids for human nutrition and is a complete protein **(Dudek, 2001)**.

As an important source of high-quality protein in the human diet, fish has been known for a long time. *Scombromorous* spp. (Kapreeta tuna-like fish) accounts for around 8-10% of Egypt's total marine fish harvest **(Abu-Tor, 2002)**. Egyptians dislike this type of fish because of its crimson black flesh and many blood veins. This means that significant quantities of raw materials are lost every year because of their susceptibility to fast deterioration. As a result, this fish must be used to produce commercial fish products **(Venugopal, 2003)**. Recently, fish processing technology has been evolving in a way that focuses on technological up-grading, diversity, and quality assurance. Due to this, there is an increased demand for seafood/seafood-based convenience foods that may be eaten or cooked right away (**Ammar and Korish *et al,* 2009**).

Fish flesh that has been mechanically deboned and cryoprotectant-treated to extend its shelf life is known as surimi. Wringing out the gel raises the concentration of myofibril protein which increases the gel's strength and flexibility. It also eliminates fat and other unwanted things such as blood, pigment and odoriferous materials. For example, shellfish analogues can be created using this feature **(Maqsood et al., 2013)**. This work carried out to study the effect of partially substitution of rice bran and soybean protein concentrates on chemical, nutritional and organoleptic properties of Kapreeta tuna-like fish (*Scombromorous Spp.)* surimi.

Materials and Methods

Materials

Freshly mixtures of generated rice bran were collected from Desouk rice milling Co., Desouk city, Kafr Elshiekh Governorate, Egypt, then were stored at 4°C in polyethylene bags before use. While soybean protein concentrate was obtained from Food Technology Research Institute, Agricultural Research Center, Giza, Egypt during the winter season of 2019. Kapreeta fish (***Scombromorous Sp***.) like tuna was purchased from the local market of Alexandria City, Egypt during the summer season of 2019. Samples were transported directly in icebox (at 5-10°C) to the laboratory of food technology department, faculty of Agriculture, Kafrelsheikh University. Rice, vegetables (green coriander and parsley), spices (black pepper), salt and sunflower oil were bought from the local market of Kafrelsheikh city. El-Gomhoria Company for Chemicals and Drugs in Egypt supplied the chemicals utilized in this investigation.

Methods

Defatting and rice bran protein concentrating

According to **Kaewka *et al.* (2009)**, defatted rice bran (DRB) was produced using RB that had been newly milled by defatting twice with hexane (1:3, w/v), followed by air drying overnight in the fume-hood, grinding in sample mill, and sieving through a 0.5-mm screen. Distilled

water was added to the defatted bran (1:10). By adding 4 ml of NaOH solution and stirring for 1 hour, the pH of the slurry was adjusted to 9.0. (12,600g, 15 min). With HCl (4 M) acid, we adjusted the pH of the supernatant protein solution to 4.5, agitated it for 30 minutes, and then left it undisturbed for cold precipitation overnight (4°C). Protein precipitates were rinsed three to four times with deionized water after carefully removing the supernatant from the solution. It was then lyophilized and pH adjusted to 7.0. Rice bran protein concentrate was the name given to the final product (RBPC).

Preparation of kapreeta fish

The samples were removed from the icebox and washed by running tap water to remove the adhering sand and silt. Then, treated by whiteness treatments by immersed in 5% H_2O_2 solutions in glass jars for 1 min to remove the blood in fish flesh after removed the head, skin and viscera. The flesh of kapreeta fish isolated from the bones, where it was minced using meat mincer (molunix mincer HV، France) (**Fadl, 2014).** The minced fish flesh used for preparing Surimi blends, as follows in Table (1). The samples of Surimi blends were packed in polyethylene bags and stored in a deep freezer (at -20 °C for 6 months) until analysis.

Frying of Surimi

Frozen Surimi blends were thawed at room temperature to studied chemical, microbiological and organoleptic properties after frying with sunflower oil (at 230-250°C) for 5-10 min compared with unfired Surimi.

Proximate chemical composition of rice bran (RB), defatted rice bran (DRB), rice bran protein concentrate (RBPC) and soybean protein concentrates.

Moisture was assessed by drying it in air oven at 105˚± 5°C until it had a uniform weight; Micro-Kjeldahl method was used to determine crude protein content using nitrogen-to protein conversion factors of 5.75 for rice bran and 5.71 soybean protein concentrate, the ether extract was carried out in a Soxhlet apparatus using petroleum ether (40 - 60°C) as the solvent. The ash content was determined by ashing a known weight of a fat-free sample in refluxing 1.25 percent (w/v) sulfuric acid and 1.25 percent (w/v) sodium hydroxide in an electric muffle at 550°C until complete ashing, and the crude fiber content was determined by digesting a known weight of a fat-free sample in refluxing 1.25 percent (w/v) sulfuric acid and 1.25 percent (w/v) sodium hydroxide. Total and available carbohydrates were calculated by difference. The procedures provided in **A.O.A.C. (2000)**. were used to determine all of the previously listed compounds. All measurements were performed in triplicate.

Determination of minerals content

Minerals were measured using a 6N HCL after wet ashing, and zinc, copper, iron, and manganese were assessed using an atomic absorption spectrophotometer (Zeiss FMD3). Potas-

Table 1 Ingredients and mixtures of Surimi blends (%).

Ingredients (gm)	No1	No2	No3	No4
Whitened kapreeta fish flesh (with 5%H_2O_2)	100	85	85	85
Rice bran protein concentrate	0	15	0	0
Soybean protein concentrate	0	0	15	-
Rice	10	10	10	10
Vegetables (green coriander and parsley)	3	3	3	3
Black pepper	1	1	1	1
Salt	1	1	1	1

Figure 1. Kapreeta (Like tuna fish)

Figure 2. Separation of fish flesh from the bone.

Figure 3. Whitening of the flesh using H_2O_2



Figure 4. Fresh and fried Surimi.

sium, sodium, and Calcium were measured using a flame photometer, and phosphorus was calculated photometrically using a phosphorus molybdate complex spectrophotometer at 650 nm and a standard curve, as reported in **A. O. A. C. (2000)**.

Amino acid analysis

Shimadzu HPLC apparatus (Shimadzu, Kyoto, Japan) was used to evaluate amino acid composition, which included Shimadzu SIL RF10Axl fluorescence detectors, a Shimadzu SCL-10Avp controller with a column oven, and a Shimadzu SIL-10ADvp auto-sampler running the CLASS-VP software (version 5.03). The column was Shimpack Amino-Na (6100mm) and flowed at 0.6 ml/min. The excitation wavelength (EX) was 348 nm, while the emission wavelength (Em) was 450 nm. Elution buffers were (A) 0.2M citrate buffer (pH 3.3), (B) 0.6M citrate+ 0.2 M boric buffer (pH10), and (C) 0.2 M NaOH. Briefly, samples were hydrolyzed using 6 N HCl/1% (w/v) phenol vapor at 110°C for 24 h in vacuo. The amino acids were treated with o-phtalaldehyde (OPA) to form OPA-derivates, which were then analysed using the above HPLC.

Determination of tryptophan

The tryptophan content of each sample was calorically measured individually using alkaline hydrolysis and p-dimethyl –amino –benzaldehyde (DMAB) according to the Miller (1967) technique. The tryptophan concentration was determined using a standard curve created under the same circumstances and quantified spectrophotometrically at 590 nm.

Nutritional quality determinations

Nutritional qualities were calculated on the basis of the amino acid profiles.

Chemical score for essential amino acids (AAS)

According to **Pellet and Young, (1980)** amino acid score is defined as milligrams of essential amino acid per gram nitrogen in tested protein divided by milligrams of essential amino acid per gram nitrogen in the reference protein reported by **FAO/WHO (1991).** The results were multiplied by 100, and the amino acid that showed the lowest proportion is called the limiting amino acid and the ratio obtained was the score.

$$\text{Chemical score} = \frac{\text{Essential amino acid/100 g tested protein}}{\text{Essential amino acid/100 g protein in FAO/WHO}}$$

Computed protein efficiency ratio (C-PER):

The computed technique for protein efficiency ratio (C-PER) was determined using the regression equations published by **Alsmeyer et al. (1974)**, which are listed below:

C-PER = -0.468 + 0.454(LEU) – 0.105(TYR).

Where: LEU and TYR: amino acids leucine and tyrosin.

Biological value (BV) of protein:

Farag et al. (1996) estimated the biological value of protein sources using the following equation:

Biological value (B.V.) = 49.9 + 10.53 C-PER**.**

The net protein value (NPV)

Calculating the net protein value was as simple as multiplying the lowest amino acid score by the protein percentage divided by 100.

NPV = (The lowest amino acid score × % protein)/ 100

The Essential Amino Acid Index (EAAI) was calculated using the method of **Labuda *et al.* (1982)** *according to the equation below:*

$$EAAI = \sqrt[n]{\frac{[\text{Lys x Threo x Val x Meth x Isoleu x leu x Phynylal x Histi x Trypt}]a}{[\text{Lys x Threo x Val x Meth x Isoleu x leu x Phynylal x Histi x Trypt}]b}}$$

Where: [lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine, and methionine]

a. in test sample and [lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine]

b. content of the same amino acids in standard protein (%), respectively. The nutritional index of the food samples was calculated using the formula below:

$$\text{Nutritional index}[\%] = \frac{\text{EAAI x \%protein}}{100}$$

Measurement of protein quality by in vitro digestibility

In order to determine the digestibility of rice bran, soybean, and whey proteins, the 4-enzyme technique provided by AOAC (1984) was modified somewhat. With the addition of 0.1 N NaOH or HCl, at 37°C, 10 ml of crude protein (6.25 mg/ml) was adjusted to pH 8. One millileter of newly produced enzyme stock solution was added to the protein suspension at 37°C for 10 minutes. As soon as the solution has been incubated for 10 minutes. We added 1.1 mg/ml of protease enzyme in water (Solution B), incubated for 9 minutes at 55°C, then for 1 minute at 37°C, and measured the pH. In order to determine protein digestibility, the following equation was used:

% Digestibility = 234.84 - 22.56 (X)

Where: X = pH after 20 min incubation.

Determination of the antioxidant activity

A modified version of **Fujinami *et al*. (2001)** technique was used to determine the radical-scavenging activity of DPPH. It was dispersed at 0.50mmol/L of DPPH in ethanol. We added diluted bran extract or L-ascorbic acid (VC), which was employed as a standard, to 1mL of DPPH solution, and the results were compared. For 20 minutes, at 25 °C, the mixture was violently shacked and kept in the dark. We used a UV-1200 spectrophotometer (Shimadzu, Kyoto) to evaluate the absorbance of the solution at 516nm in comparison to 4mL of 50 % (v/v) ethanol in 4mL of 1mL of diphenhydramine (DPPH) solution. The radical scavenging activity of the diluting extraction or VC liquid was determined as the quantity of extract required to decrease the original DPPH concentration by 50%:

Radical scavenging activity = (A - B +C) / A×100 (%)

Where: A represents the starting absorbance of the blank, B represents the absorbance of the diluted extract and DPPH solution mixture at 20 minutes, and C represents the diluted extraction absorbance without the DPPH solution. The radical scavenging activity was quantified in mmol-VC/g-bran as VC equivalent antioxidant capacity. For each bran extraction, triple preparations were carried out.

Microbiological tests

Samples were prepared using the recommended methods for the microbiological examination of food published by **A.P.H.A (American Public Health Association) (1971*).*** Fish samples were aseptically taken using sterilized knives and placed in sterile containers. One gram of samples were emulsified in 99 ml sterile distilled water in a sterile mechanical blender cup. Every determination was done in triplicate. Total viable bacterial counts per gram of Surimi were determined using standard techniques on plate count agar (Difco). Incubation was carried out for 48 hours at 32°C according to **Deng *et al*. (1976).** Mold and yeast counts were determine by plating on acidified potato dextrose agar (Difco), and incubating duplicate plates at 25°c for 5 days (**Nottingham,1971**). Coliform bacterial counts were determined by plating on crystal violet neutral red agar. The plates were incubated at 25 °C for 4 – 5 days. The colonies were count and reported as coli forms per gm of flesh **(Nottingham, 1971*).***

Organoleptic properties

Organoleptic properties were evaluated for color, flavor, texture, and overall acceptability during processing steps. A group 10 as described by **Teeny and Miyauchi (1972*).***

Statistical analysis

One-way ANOVA was used to analyse all the data, with Sigma Stat (v.3.5. Systat Software Inc.). Duncan's novel multiple range test found that there was a significant difference between treatment means at the P 0.05 level **(Steel and Torrie, 1980)**.

Results and discussion

Chemical composition of rice bran, soybean, and whey protein concentrate:

The raw rice bran, defatted rice bran (DRB), rice bran protein concentrate (RBPC), soybean protein (SP), and whey protein concentrate (WP) were chemically analyzed for their contents of moisture; protein; ether extract; ash; crude fiber. Total and available carbohydrates were calculated by difference. The obtained data are presented in Table (1). It could be noticed from the results in Table (1) that the moisture content of RBPC and SP were 3.91 and 7.30, respectively. Raw and defatted rice bran had 9.05% moisture content for each one. Concerning the crude protein content, results disclose that the protein content of RBPC had the highest amount of protein, (72.9%). It is also noted that the defatted rice bran had significantly the highest ash content (11.30%). On the other hand, the carbohydrate and crude fiber contents indicated that there were significant differences between the two sources of proteins. The obtained results are in agreement with the results of **(Supathra *et al*., 2008, Gul *et al*., 2015)** they reported that full fat rice bran were contained 8.5-12.6% moisture, 4.2-7.7% ash, 8.0-18.9% ether extract, 8.8-15.2% protein, 22.2-44.8% total carbohydrates and 18.3-30.5% for total dietary fiber (on dry weight basis).

Table (1): Chemical composition of rice bran and soybean protein concentrates (g/100g dry matter).

Soybean protein concentrate	Rice bran			Components
	Protein concentrate	Defatted	Raw	
7.30[b]	3.91[d]	9.05[c]	9.05[a]	Moisture
92.70[b]	96.09[c]	90.95[d]	90.95[c]	Dry Matter
48.10[a]	72.90[a]	17.24[d]	14.29[c]	Crude protein
6.02[b]	0.00	0.22	17.10[a]	Ether extract
5.83[c]	4.47[d]	11.30[b]	9.37[a]	Ash content
11.50[b]	0.02[d]	18.44[a]	15.29[a]	Crude fiber
40.05[c]	22.63[e]	71.24[c]	59.24[b]	Carbohydrate *
28.22[d]	22.61[e]	52.80[a]	43.95[c]	Available carbohydrate **

Values followed by different letter in row are significantly different at $p \leq 0.05$.

Each value is an average of three determinations.

* Total carbohydrate was calculated by subtracting protein, ash, and ether extract (%) from the total mass of 100.

** Available carbohydrate obtained by subtracting crude fiber from total carbohydrate.

Mineral contents of rice bran and soybean protein concentrates

Mineral contents of rice bran, soybean protein, and whey protein concentrate were analysed and the results are present in Table (2). The data in the mentioned table clearly shown that phosphorus (680 mg/100) and potassium (2410 mg/100g) were the predominant minerals of rice bran protein concentrate and soybean protein concentrates, respectively. On the other hand, calcium (38 mg/100g) and sodium (6.8 mg/100g) in rice bran protein concentrate and sodium (123 mg/100g) in soybean protein showed low amounts. The obtained results also revealed that the three different protein sources used in this study may provide a significant amount of minerals to meet the human mineral requirements (Recommended Dietary Allowance). However, only the whey protein concentrate showed a ratio Ca : P more than 1.0, but the other two types of proteins showed avery low Ca : P ratios, (1 : 17.89 and 1 : 197 for rice bran and soy protein concentrates, respectively).

Amino acid composition

The amino acids composition of rice bran protein concentrate (RBPC) is given in Table (3). Besides the amino acid composition of soybean protein concentrate (SPC). Data in Table (3) show the amino acid composition of RBPC and SPC, along with the professional pattern recommended by **FAO/ WHO (1991)** for school children (10-12 Yr). All the three different types of protein, namely, of RBPC, SPC and WPC, as indicated in Table (3) were rich in essential amino acids and met human requirements for all the essential amino acids. Protein of RBPC and SPC contained 51.39 and 53.60g essential amino acid/100g protein respectively. These values are higher than that recommended by FAO/WHO (1991) pattern for children of 10-12 years (24.10 EAA/100g protein. Tryptophan had the lowest concentrations of essential amino acids and valued 1.96, 2.12 and 2.07, followed by sulfur containing amino acids (methionine + cystine) which amounted 3.76, 4.62 and 5.43 g/100g protein), respectively. Data presented in the same table cleary indicate that glutamic acid (8.20 and 15.02 g/100g protein), followed by aspartic acid (6.13 and 9.78 g /100g protein) were the most abundant amino acids and form a major part of amino acids in RBPC and SPC. The ratio of essential amino acids (EAA) to non-essential amino acids (NEAA) ranged between 1.16 for the SPC and 1.33 for the RBPC.

Table (2): Minerals content of rice bran and soybean protein concentrates (mg/100g on dry weight basis).

Mineral content (mg/100g)	Rice bran protein concentrate	Soybean protein concentrate
Macro-minerals		
Calcium (Ca)	38[c]	377[c]
Sodium (Na)	6.8[d]	123[d]
Potassium (K)	430[b]	2410[a]
Phosphorus (p)	680[a]	743[b]
Trace elements		
Zinc (Zn)	3.2[e]	5.5[f]
Copper (Cu)	0.8[f]	4.8[f]
Iron (Fe)	0.7[f]	15.0[e]
Manganese (Mn)	4.4[e]	12.2[e]

*Values followed by the same letter in row are not significantly different at $p \leq 0.05$.

Values followed by different letter in row are significantly different at $p \leq 0.05$.

In conclusion, it could be observed that some differences in the amino acid contents were noted between the three different types of proteins. However, the content of all essential amino acids was higher than those recommended by FAO/ WHO pattern. And, it is the essential amino acid content that counts for the quality of protein and its capacity to satisfy the needs for essential amino acids.

Chemical score of essential amino acid

Chemical score provides an estimate of the nutritive value of a protein by comparing the levels of essential amino acids between the test protein and those of the FAO/WHO pattern for children of 10- 12 years old. The essential amino acid scores of protein from RBPC and SPC are shown in Table (4). The data in Table (4) indicate that all essential amino acids, except the histidine in whey protein concentrate, of the three different proteins are in excessive amounts. It is the young children who suffer from malnutrition, and protein resembling the scoring pattern in composition should provide essential amino acids in excess when they are consumed by the older age group. The results in Table (4) agreed with other findings by **(Bhaskar *et al*. 2008; Ovissipour *et al*. 2009).**

Nutritional quality of protein

Table (5) shows the data related to the protein efficiency ratio (C-PER) and biological values of RBPC and SPC, compared with casein as a reference protein, along with net Protein Value (NPV), essential amino acid index (EAAI), nutritional index (NI) and in vitro protein digestibility.

Methods based on in vitro (chemical and amino acids bioassay) for assessment of protein quality are important. The digestibility is important criterion that determine the availability of physiologically active amino acids and peptides and is affected by processing treatments **Dudek (2001)**. The true digestibility values of the proteins of RBPCs and the standard protein (casein) found are different from each other. The result in Table (5) indicated that

Table 3. Amino acid composition of rice bran and soybean protein concentrates (g amino acid/100g protein) (on dry weight basis).

FAO/WHO/ 1991 (10-12yr)	Soybean protein conc.	Rice bran protein conc.	Amino acids (g/100g protein)
			Essential amino acids (EAA)
2.8	4.26	4.16	Threonine
2.5	6.16	6.15	Valine
2.8	8.16	7.80	Isoleucine
4.4	7.62	7.43	Leucine
4.4	8.77	8.72	Lysine
1.9	3.28	3.90	Histidine
2.2	2.31	1.88	Methionine
	2.31	1.88	Cystine
	4.62	3.76	Total sulfur amino acid
2.2	5.32	4.84	Phenylalanine
	3.29	2.67	Tyrosine
	8.61	7.51	Total aromatic amino acids
0.9	2.12	1.96	Tryptophan*
24.10	53.60	51.39	Σ1=Total EAA
			Non-essential amino acids (NEAA)
	4.45	5.39	Proline
	9.78	6.13	Aspartic
	3.72	2.26	Serine
	15.02	8.20	Glutamic
	3.66	3.26	Glycine
	3.74	6.13	Alanine
	6.03	7.24	Arginine
	46.40	38.61	Σ_2= Total NEAA
	100	90	$\Sigma = \Sigma_1 + \Sigma_2$
	1.16	1.33	Σ_1 / Σ_2
	47.56	49.67	$A = \Sigma_1 \Sigma_1 + \Sigma_2$

Σ1= sum of essential amino acids; Σ_2= sum of non-essential amino acids.

*Methionine + cysteine (SAA); Phenylalanine + tyrosine (AAA).

[1]Amino acid scoring patterns: Theoretical standard of FAO/WHO/ UNU essential amino acids for children between 10 and 12 years old (FAO/WHO/ UNU, 2007).

- Tryptophan was determined colorimetrically.

Table 4. Chemical score (CS)* of essential amino acids of rice bran and soybean protein concentrates with respect to the required pattern recommended by FAO/WHO (1991).

Soybean protein concentrate		Rice bran protein concentrate		FAO/WHO recommended pattern for 10-12 yr children	Essential amino acids
CS	g/100g protein	CS	g/100g protein		
.36	4.26	1.49	4.16	2.8	Threonine
2.46	6.16	2.46	6.15	2.5	Valine
2.91	8.16	2.79	7.80	2.8	Isoleucine
1.73	7.62	1.69	7.43	4.4	Leucine
1.99	8.77	1.98	8.72	4.4	Lysine
1.73	3.28	2.05	3.90	1.9	Histidine
2.1	4.62	1.71	3.76	2.2	Methionine+cystine
3.91	8.61	3.41	7.51	2.2	Phenylalanine+tyrosine
2	2.12	2.18	1.96	0.9	Tryptophan

* Chemical score was calculated as a percentage of FAO/WHO, 1991 essential amino acids.

* * Limiting amino acid

Table 5. Estimation of nutritional quality of rice bran and soybean protein concentrates and casein, based on their amino acid composition.

Casein	Soybean protein concentrate	Rice bran protein concentrate	Parameters
3.35	2.65	2.62	Computed protein efficiency ratio (C-PER)
85.20	77.80	77.49	Biological value (B.V)
0.78	1.02	1.37	Net protein value (NPV)
1.55	1.81	1.75	Essential Amino Acid Index (EAAI)
1.16	0.87	1.27	Nutritional index (NI) (%)
95.30	90.50	85.20	In vitro protein digestibility

Table 6. DPPH radical scavenging (mmol-VC/g) of rice bran and soybean protein concentrates and casein, based on amino acid composition.

DPPH Radical scavenging (mmol of ascorbic acid (VC/g)	Parameters
0.35^{a}	Rice bran protein concentrate
0.34^{b}	Soybean protein concentrate
0.09^{c}	Casein

Values superscripted with dissimilar letters (a, b and c) are significantly different ($p < 0.05$).

the highest digestibility value using trypsin was found for RBPC (85.20%) in comparisons by SBPC (90.50%). Moreover, the digestibility found for casein was 95.30% **Mune *et al.* (2011)**.

DPPH radical-scavenging activity of rice bran protein concentrates (RBPCs)

Some of the chemicals in rice bran have been found to have antioxidant action. Vitamin E (Tocopherols and Tocotrienols), phytosterols, oryzanol, and amino acids, notably arginine, histidine, cystein, and methionine, are examples of these substances **(Sereewatthanawut et al, 2008)**. It is commonly acknowledged that the DPPH free radical is a good model for assessing the antioxidant properties of diverse samples. The ability of antioxidants to scavenge DPPH radicals was linked to their ability to donate hydrogen. It measures the ability of substances to scavenge free radicals or donate hydrogen atoms. In contrast to the extremely reactive peroxyl and hydroxyl radicals that are involved in lipid peroxidation and tissue damage in biological systems, DPPH is a stable synthetic radical (**Intarasirisawat *et al.,*** 2012). Chemicals that can neutralize free radicals would do so by giving them one of their electrons, which would prevent them from spreading. In this work, RBPCs were evaluated for their ability to scavenge stable DPPH radicals. This was followed by SPC (0.34mmol) and then WPC (0.34mmol), with casein having the lowest total antioxidant capacity and RBPC having the highest total antioxidant capacity (Table 6).

Chemical composition of unwhitened and whitened kapreeta fish flesh (Like tuna) (on dry weight basis)

The results in Table (7) indicated that the chemical composition differs between unwhitened and whitened

Table 7. Chemical composition and minerals content of fresh and whitened Kapreeta fish (like tuna) flesh (on dry weight basis).

Characteristics	Kapreeta fish (fresh)	Kapreeta fish (whitened with 5%H2O2)
Moisture %	77.80[a]	67.14[b]
Crude protein %	78.99[b]	82.30[a]
Ether extract %	6.80[b]	8.02[a]
Ash %	6.46[b]	6.77[a]
Carbohydrates	7.75[a]	2.91[b]
PH	6.92[b]	6.98[a]
Minerals content (mg/100g)		
Ca	35.42[a]	35.35[b]
K	11.80[b]	12.60[a]
Na	25.40[a]	20.70[b]
P	18.40[b]	19.90[a]
Zn	28.10[a]	28.02[b]
Fe	109.22[A]	100.01[B]

Means within the same row of different letters are significantly different at ($P < 0.05$).

Table 8. Chemical composition of unfried and fried Surimi blends prepared from whitened Kapreeta fish flesh supplemented with 15% level of soybean, whey and rice bran protein concentrates (On dry weight basis).

Characteristics (%)	Surimi blends					
	No1 (Control)		No 2 (Added 15% rice bran protein concentrate)		No3 (Added 15% soybean protein concentrate)	
	Unfired	Fried	Unfired	Fried	Unfired	Fried
Moisture	65.58	42.35	62.53	43.24	63.85	42.06
Crude protein	65.67	64.31	62.27	60.31	60.51	59.50
Ether extract	5.41	11.38	4.98	10.65	4.60	10.30
Ash	6.20	5.80	6.68	6.23	6.82	5.64
Carbohydrates	22.72	18.51	26.07	22.81	28.07	24.56

kapreeta fish. It could be observed some changes as affected by whiteness treatment. Crude protein, ether extract and ash content were higher in whitened kapreeta fish, but moisture content and carbohydrate contents were low. The results in the same Table indicated that the unwhitened kapreeta fish had some higher minerals Na and Fe than the unwhitened kapreeta fish and these results agreed with those of **(Abu-Tor, 2002a; Chaijan *et al.*, 2004; korish *et al* 2008 and Ammar and korish, 2009)**. Where they reported that fish whiteness causes a decrease of moisture, carbohydrates, and some minerals but increases the concentration of crude protein, ether extract, and ash.

Chemical composition of Surimi blends prepared from whitened kapreeta fish flesh supplemented with 15% level of rice bran and soybean protein concentrate

The results cleared in Table (8) showed that the chemical composition of fried Surimi blends prepared from whitened kapreeta fish flesh with (5% H_2O_2). Frying process had noticeable effects on the chemical composition of fried Surimi blends. The moisture content of unfried Surimi blends were decreased by heat treatments of frying because of evaporated amounts of moisture during frying. The previous results agree with **Ammar (1999).** As for crude protein, there was a little decrease after the frying process, this may be due to the effect of heat treatment which might have caused the loss of some nitrogenous substances with separated fluids as well as volatilization in the form of amines and other volatile nitrogen substances**.** Ether extracts were increased by the frying process however it was notably that fried Surimi blends in sunflower oil. Ash content was decreased in all samples after frying. It was noticed that there is no difference between ash content in fried Surimi blends. The carbohydrate content showed a higher level in unfried Surimi blends than in the fried ones. Finally, from the presented results in Table (8) it could be observed that protein content and ash of prepared samples were in the same trend as control but carbohydrate was higher than control. The previous results are in agreement with those of **(Ammar, 1999 and Fadl, 2014)**.

Table 9. Organoleptic properties of fried Surimi blends prepared from whitened kapreeta fish flesh supplemented with 15% level of rice bran and soybean protein concentrates.

Fried Surimi blends	Organoleptic properties score					
	Appearance	Color	Odor	Texture	Taste	Overall acceptability
No1(Control)	8	8	7	7	8	7.60
No 2 (Added 15% rice bran protein concentrate)	8	9	8	9	8	8.40
No3 (Added 15% soybean protein concentrate)	8	9	8	7	9	7.80

Organoleptic properties of fried Surimi blends prepared from whitened kapreeta fish flesh supplemented with 15% level of rice bran and soybean protein concentrates:

The results showed in Table (9) show that the organoleptic score of fried Surimi blends (No 1) (control) prepped from whitened kapreeta fish flesh (control) were 8, 8, 7, 7, 8, and 7.60 for appearance, color, odor, texture, taste, and overall acceptability. While the organoleptic score of fried Surimi blends prepared from whitened kapreeta fish supplemented with 15% level of rice bran and soybean protein concentrates were higher than control for the same properties and overall acceptability were 8.40 and 7.80 for rice bran and soybean. Our results cleared that, the addition of 15% rice bran protein concentrates to the kapreeta fish during Surimi making improved the organoleptic characters of the Surimi **Ammar and Korish (2009*)***.

Microbiological properties of unfried and fried Surimi blends supplemented with 15% level of rice bran and soybean protein concentrates.

Table (10), cleared the microbiological qualities of unfired Surimi blends (fresh) prepared from whitened kapreeta fish flesh. Total viable bacterial counts, molds & yeast, and coliform were counted. From these results, we can notice that Surimi blends No 2 were the lowest counts after steam blanching as a sequence of heat temperature. The total viable bacterial count showed a higher level in fresh control kapreeta fish Surimi.

Our results agreed with those of **(Kittikun *et al*., 2012*)*** who reported that the tuna-like fish Surimi does not contain any coliforms, especially *E. coli* due to the water in which tuna-like fish bred it is commonly free from *E. coli* but the most bacterial isolates from Surimi includes *Enterobacter* sp., and *Providencia* sp. Table (10) cleared the differences of microbiological counts of fried Surimi blends prepared from whitened kapreeta fish flesh. The total viable bacterial count in fried Surimi blends No 1, No 2, and No 3 were 4.00×10^3, 3.3×10^3 and 4.2×10^3, respectively. While, the molds and yeast counts in these samples were 0.78×10^2, 1.0×10^2 and 0.58×10^2 respectively. The results also cleared that, the coliform group not recorded in these samples. Finally, we can conclude that samples prepared with 15% of two kinds of added protein were less in counted microbiological qualities in both. Our results agreed with those of **Ammar (2004*)*** who reported that frying the fish destroys most bacteria, molds, and yeasts in the fish.

Conclusions

From the previous results, it could be concluded that all three types of protein, the cereal one, the legume, and the animal source are considered important sources of protein, ash content, carbohydrate, and minerals especially potassium phosphorus, iron, and manganese. The content of all essential amino acids was higher than the recommended by FAO/WHO pattern for 10-12 year children. The nutritional quality of the three different sources of protein was highly

Table 10. Microbiological qualities of fresh (unfried surimi blends (prepared from whitened kapreeta fish flesh (with 5 % H_2O_2).

Surimi blends	Process	Microbiological qualities		
		Total viable bacterial counts	Molds and yeast counts	Coliform bacterial counts
No1(Control)	Fresh	7.2×10^3	5.8×10^3	2.1×10^2
	Fried	2.00×10^2	0.78×10^2	Nil
No 2 (Added 15% rice bran protein concentrate)	Fresh	4.8×10^3	1.20×10^2	Nil
	Fried	1.3×10^2	1.0×0^2	Nil
No3 (Added 15% soybean protein concentrate)	Fresh	5.0×10^3	1.30×10^2	Nil
	Fried	1.55×10^2	0.50×10^2	Nil

Value are Means ± Standard Error. Means within the same row of different letters are significantly different at ($P < 0.01$).

comparable to those of antioxidant activities showed closed values and was higher than that of casein, a standard protein source. Accordingly, it could be concluded that rice bran, soybean, and whey protein concentrates can be recommended for preparing some food products. The tuna-like fish is of poor nutritive value and is not appreciated among consumers, so it can be utilized for the production of fish products as the experiment source of protein. Then using it for Surimi making with the addition of 15% of different sources of protein concentrates will improve the feeding and nutritive quality of this fish.

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