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Study on the effect of freshness of raw materials on the final quality of fish meals

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Two types of fish meals namely fresh fish meal (FFM) and stale fish meal (SFM) were prepared using oil sardine (*Sardinella longiceps)* by wet reduction method. FFM was prepared from fresh fishes, and SFM was prepared from oil sardines stored in refrigerator for 3 days at 8° C. The qualities of these two fish meals were studied by assessing the nutrient contents and quality indicators. The quality of stored fish meals was also studied with Ethoxyquin antioxidant for 2 months. When compared to stale fish meal, fresh fish meal retained a better proximate composition with essential amino acids, fatty acids, vitamin, and minerals. It also had acceptable limits of microbial and biochemical quality indicators. This study demonstrates that fresh fish meal prepared by wet reduction method has good qualities due to the hygienic processing of fresh fishes. The biochemical constituents of fish meal prepared in this method are better retained than in other methods. Fish meal with good quality has a longer shelf life of about 3 months, and with the addition of antioxidants like ethoxyquin shelf life can be extended even up to 6 months.

[Keywords: Antioxidant; Fish meal; Frozen, Oil sardine; Refrigerated stored fishes]

Introduction

Fishes used in the production of fish meal is known as feed fish and it is comprised of pelagic fishes such as anchovies, sprats, mackerels, herrings and sardines[1]. It has little market value and in many places it is simply discarded or unhygienically sand-dried and used as poultry feed. In some developing countries like China, Thailand and Vietnam fish meal is used as a protein source for pig[2], poultry and aquatic animals[3]. The production of fish meal and oil is the predominant method of processing the world's non-edible fish. Fish meal is an essential dietary ingredient in the industrial aqua feeds[4]. Further, fish meal provides high quality protein in addition to long-chain omega-3 fatty acids, like Decosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). It is also a valuable source of minerals and trace elements. The chemical compositions vary greatly depending on the species of fish used for the production of fish meal[5]. Approximately 85 % of aqua-cultured species depends on fish meal for feed[6]. Around 6.5 million tons of fish meal and 1.2 million tons of fish oil is produced annually worldwide[7]. In tropical areas, normally in fish landing centers, the fish processor highly concentrate on commercially high valued fishes and they are iced immediately and properly. But the low value fishes are preserved with slightly sprinkled ice, hence in tropical areas it is a very hot subject in fish meal quality.

Approximately, 4 to 5 tons of whole fishes are required to produce 1.5 ton of dry fish meal[8]. Utilization of fresh fish in the preparation of high-quality fish meal is much limited because its species composition is variegated[9]. About 94,000 metric tons of oil sardine fishes are used to produce 9,235 tons of farm fishes in Malaysia[10]. But in India, utilization of fresh fish is poor[11]. In the past, the unutilized fishes were broken up into small pieces to feed the cultured species. Only 30-50 % of the fishes were consumed and the rest is left to decompose creating localized pollution and degradation of water quality. Then only the formulated feed was developed using the powdered fish meal. Good quality fish meal demands a higher price than other high protein feedstuffs[12]. Peru is one of the leading fishmeal producing countries and it supplies almost one-third of the total world fish meal. Other principal fish meal producing countries are Chile, China, Thailand, U.S.A., Iceland, Norway, Denmark and Japan. Increasing production of aquatic-farmed species has created a demand for high-quality fish meals[13]. High-quality fish meal can be produced from fresh fishes by processing under low temperature.

Spoiled raw material when overheated may lead to the production of toxins in fish meal[14]. Attempts to produce high-quality fish meal with low-cost fish supplements have not been successful[15]. Preserving excess landing of fishes in the traditional way remains a problem in rainy climates and the price level in these regions are low and skill to operate hi-tech equipment is almost absent. Local people cannot afford to buy high-tech processing equipment's. Processing of fish meal using low-cost fishes and low-cost equipment is essential to produce a low-cost fish meal. In the present study, fish meals were developed by wet reduction method from the frozen stored fresh fishes and their qualities were compared with refrigerated stored fishes to clarify the preparation methods and qualities of raw materials and their impact on the final quality of the fish meal.

Materials and Methods

Collection of raw materials

Fresh samples of fish were collected from the fish landing centre and transported to the laboratory. Half of the samples were stored in a frozen condition (-4 ^{o}C) and the other half was stored at refrigerated temperature (8 ^{o}C). After 48 hours of storage, the samples were used for further processing of fish meal.

Fresh fish meal (FFM)

Fishmeal was prepared by the wet reduction according to the method of Kamasastri and Ramananda Rao[16]. Five kg of fish is needed to prepare 1 kg of fish meal. Five kg of aggregated trash fish was steamed for 15 minutes to soften the flesh and bones, to coagulate the protein and fat depots, and to liberate oil and water. Then it was pressed to remove a large fraction of oil and water from the solid parts of the fishes. The oil and water is called stick water and the solid portion is known as press cake. Stick water was concentrated in a water bath (70 ^{o}C) to evaporate the water, and the remaining oil was added to the press cake and it was dried for 5 to 6 hours at 60^{o}C in a hot air oven and then was powdered.

Stale fish meal (SFM)

SFM prepared from sardine fishes was stored under refrigerated temperature. For this, 5 kg of sardine was stored for 48 hours in refrigerated temperature (8 ^{o}C) and it was processed by the wet reduction method as mentioned above.

Quality analysis of fish meals

The fish meals were analyzed for their proximate composition, which is as follows: moisture[17], protein[18], lipid[19], total ash and acid insoluble ash and fibre[17]. Carbohydrates as nitrogen free extract (NFE) were calculated by difference. The non-saponifiable lipids such as cholesterol were analyzed spectro-metrically according to the method of Zlatkis et al.[20]. Caloric content was calculated by multiplying the concentration of various nutrients with conversion factors namely 4.15, 9.40 and 5.65 of carbohydrate, lipid and protein, respectively[21]. The caloric values were expressed as calories per gram (cal / gm) on dry weight basis. Salt content of the fishmeal was determined using silver nitrate. Non-protein nitrogen especially urea was determined qualitatively using mercuric-potassium iodide alkaline solution[22] with urease and urea standard. The protein solubility of the fishmeal was determined from the supernatant[23]. TVB-N and TMA-N were determined according to the procedure of Siang and Kim[24] by using Conway micro diffusion unit. Histamine content of the fish meal was determined using perchloric acid, n-butanol and O-ophthaldehyde, and the intensity was determined using a spectrometer at wavelength of 439 nm[17]. Free fatty acid (FFA) was measured by titration with NaOH and expressed as percent oleic acid equivalent[25]. Thiobarbituric acid (TBA) value was determined through colorimetric estimation of malonaldehyde[26] and its value expressed as mg malondialdehyde kg^{-1} fish meal. The peroxide value (PV) was determined by titrating the iodine liberated from potassium iodide with standardized 0.01 N sodium thiosulphate solution[27] and it was expressed as milli-equivalents of free iodine kg-1 of lipid. Fatty acid composition of the meals was analysed using gas chromatography[28]. Amino acids were analysed by the Pica-Tag method described by Waters Chromatography Division, which involves precolumn derivatization with phenyl isothiocyanate and separated by high-performance liquid chromatography (HPLC) on a reversed-phase column (Waters Pica-Tag column for total amino acids)[29].

Storage studies

A part of the two fish meals were divided into two equal portions and one portion was treated with Ethoxyquin (150 ppm / kg) and the other was untreated. Both the fish meals were stored at ambient conditions for six months. The moisture

content, bacterial count, fungal count, TMA-N, TVB-N, FFA, PV and TBA were analyzed at monthly intervals.

Results

The results of the biochemical analysis of composition and the study on quality indicators of the experimental fish meals are presented in Table 1. All chemical parameters of the fish meals were affected significantly (P = 0.05) by the processing method used. The highest moisture content was recorded in SFM (16.54 %) and it varied significantly from FFM (5.80 %). The protein content was higher in FFM (69.75 %) and significantly lower in SFM (32.95 %). The variation between the lipid contents of SFM (4.83 %) and FFM (9.9 %) was also significant. Fiber contents of the fish meals were not higher than the acceptable limit of 3-5 %.

The NFE of carbohydrate was significantly high (31.0 %) in SFM, whereas the FFM had significantly very low (4.07 %) NFE. The total ash content was high in SFM (14.68 %). The cholesterol content of FFM was higher (65.4 mg/100g) but it was less (19.6 mg/100g) in the SFM. The average total caloric value of the biochemical components utilized as energy sources viz. lipids, proteins and carbohydrates were 361.76 cal/g and 203.11cal/g on dry weight basis for FFM and SFM, respectively.

Table 1 — Chemical composition of the experimental fish meals

Biochemical parameters	FFM	SFM
Proximate composition		
Moisture (%)	5.80±0.26^a	16.54±0.46^b
Protein (%)	69.75±1.54^b	32.95±2.07^a
Lipid (%)	9.9±0.10^b	4.83±0.19^a
Fibre (%)	0.5±0.20^a	1.1±0.16^b
Nitrogen free extract (%)	4.07±0.07^a	31.0±2.64^b
Total ash (%)	11.48±0.36^a	14.68±0.07^b
Cholesterol (mg/100g)	65. 4±1.4^b	19.6±1.65^a
Calorie content (cal/ gm)	361.76±6.65^b	203.11±4.38^a
Quality indicators		
Salt (%)	0.9±0.13^a	3.9±0.36^b
Acid insoluble ash (%)	00±00^a	12.71±0.33^b
Non protein nitrogen	Absent	Absent
Urea (%)	Absent	Absent
Protein solubility (%)	00±00^a	40.6±0.35^b
TVB-N mg N/100g	19.9±0.80^a	93.0±1.00^b
TMA-N mg N/100g	1.22±0.08^a	35.56±2.71^b
Histamine (mg/100 g)	1.03±0.07^a	9.2±0.10^b
FFA Oleic acid/g	1.24±0.03^a	9.33±0.19^b
Peroxide Value (meqo$_2$/kg fat)	00±00^a	9.52±0.23^b
TBA (malondialdehyde mg/1000g)	0.034±0.008^a	3.5±0.55^b

The salt content were observed low (0.9 %) in FFM whereas in SFM it was higher (3.9 %). Acid insoluble ash was completely absent in FFM, whereas SFM had a higher level of it (12.71 %). The qualitative analysis showed the absence of non-protein nitrogen (NPN) and urea in the fish meals. Protein solubility indicates protein degradation of fish meal. Soluble protein was absent in FFM but it was found in significantly high quantities in SFM. The values of TMA-N and TVB-N were used to determine the spoilage level of fish meal and both were found to be above the acceptable limit only in SFM (35.56 and 93.0 mgN/100g). biogenic amine such as histamine shows significant differences in FFM and SFM and the values were 2.60 and 9.20 mg/100g, respectively. The lipid hydrolysis measured by FFA value was above the acceptable limit in SFM. The primary lipid oxidation of peroxide value too was higher in SFM, and it was completely absent in FFM. The secondary lipid oxidation products of aldehyde and other volatile compounds of thiobarbituric acid reactive substances (TBARS) were found in SFM (3.50 mg MDA/kg), whereas in the FFM it was found in lower amounts (0.034 mg MDA/kg).

The fatty acid composition of the fish meals is shown in Table 2. The levels of total saturated fatty acid (SFAs) in FFM and SFM were 41.23 and 12.579 %. Palmitic acid (C16:0) was found to be the predominant SFA. The long-chain saturated fatty acid stearic acid (C18:0) was the second predominant saturated fatty acid found in the fish meals. The major monounsaturated fatty acids were palmitoleic acid (C16:1) and oleic acid (C18:1 ω9), of which oleic acid was dominant in the two types of fish meals. The

Table 2 — Fatty acid composition of five different fish meals prepared

Fatty acids (%)	FFM	SFM
Butyric acid (4:0)	1.1±0.05^b	0.2±0.17^a
Caproic acid (6:0)	1.95±0.10^b	00±00^a
Capric acid (10:0)	4.9±0.20^b	2.0±1.00^a
Lauric acid (12:0)	2.9±0.17^b	0.079±0.010^a
Myristic acid (14:0)	5.2±0.32^b	2.6±0.36^a
Palmitic acid (16:0)	19.8±1.00^b	5.0±2.00^a
Stearic acid (18:0)	5.38±0.31^b	2.7±0.30^a
Palmitoleic acid (MUFA) 16:1n-7	5.1±0.22^b	3.1±0.13^a
Oleic acid (MUFA) 18:1n-9	20.05±2.25^b	2.0±1.00^a
Linoleic acid (PUFA) 18:2n-6	10.5±0.50^b	00±00^a
Linolenic acid (PUFA) 18:3n-3	2.5±0.49^b	00±00^a
Arachidonic acid (PUFA) 20:4n-6	0.9 ±0.13^b	00±00^a
Eicosapentaenic acid (PUFA) 20:5n-3	11.8±0.26^b	2.0±1.00^a
Docosahexaenoic acid (PUFA) 22:6n-3	19.1±1.68^b	0.9±0.36^a

highest amount of oleic acid was found in FFM. The contents of polyunsaturated fatty acids (PUFAs) were 2.9 % in SFM, and 44.8 % in FFM. The average score of linoleic and linolenic acids was higher in FFM but absent in SFM. Arachidonic acid, a polyunsaturated fatty acid, was absent in the case of SFM. Of the total PUFA the concentrations of n-3 PUFA in FFM (33.4 %) were higher than those of n-6 PUFA (11.4 %). Of the ω-3 PUFA only EPA and DHA contents were the highest in FFM (30.9 %) followed by SFM (2.9 %).

There were significant differences in the amino acid compositions of the fish meals (Table 3). A total of 18 amino acids were detected, of which 10 were essential amino acids. The amount of essential amino acids in all the analyzed samples was greater in FFM (56.08 %) than in the standard protein (32 g/ 100 g protein)[25] and the amount of total amino acid per kilogram dry weight was the lowest (10.90%) in SFM. In the case of hygienic FFM, glutamic acid, aspartic acid, alanine, glycine, arginine, leucine, lysine, methionine and proline were detected in higher amounts, while glutamic acid is one of the amino acids observed to be in higher proportions in stale fish meal.

The vitamin content of the fish meals is presented in Table 4. The results indicate that fish meals have more fat soluble vitamins than water soluble ones because the prepared fish meal is a rich source of lipid. The contents of vitamins in fish meal prepared from fresh fish were observed within the range of about 7.00 mg/kg (riboflavin B2) to 108.22 mg/kg (inositol); and the measure of vitamins A, D, E and K ranges from 10.20 IU (vitamin K) to 152.34 IU (vitamin D). The quantities of vitamins in SFM ranged from about 1.00 mg (riboflavin B2) to 25.00 mg (ascorbic acid), whereas the respective values of vitamins A, D, E and K were 12.23 (IU), 43.11 (IU), 9.00 (IU) and 4.00 (mg). In general, the vitamin content of FFM was better than SFM.

The estimated quantities of the major minerals and trace elements of the samples are shown in Table 5. Calcium was the most abundant mineral present in the fishmeal, with 116.70 µg/gm in FFM and 62.14 µg/gm in SFM. The phosphorus (69.36 µg/gm) and sodium contents (83.40µg/gm) too were high in FFM. Potassium content was more in FFM (105.6 µg/gm). The chlorine content of the FFM was high (0.2122 µg/gm), while SFM shows only a trace amount of chlorine. The magnesium content was high in FFM at 89.86 µg/gm and low in SFM (30.36 µg/gm). The sulphur content of the SFM and

Table 3 — Amino acid composition of five different fish meals prepared

Amino acids (mg/100g)	FFM	SFM
Aspartic acid	9.3±0.39^{b}	3.66±0.13^{a}
Glutamic acid	13.6±1.50^{b}	5.67±0.44^{a}
Serine	4.6±0.30^{b}	1.86±0.11^{a}
Glycine	7.6±0.26^{b}	1.75±0.35^{a}
Histidine (EA)	3.3±0.43^{b}	0.20±0.04^{a}
Arginine (EA)	7.0±1.00^{b}	0.39±0.10^{a}
Threonine (EA)	3.2±0.20^{b}	0.80±0.25^{a}
Alanine	7.4±0.36^{b}	1.47±0.14^{a}
Proline	5.3±0.54^{b}	0.6±0.25^{a}
Tyrosine	2.5±0.57^{b}	1.1±0.01^{a}
Valine(EA)	4.3±0.05^{b}	0.11±0.03^{a}
Methionine (EA)	13.1±0.36^{b}	3.36±0.16^{a}
Cysteine	0.6±0.15^{b}	0.01±0.008^{a}
Isoleucine (EA)	4.2±0.10^{b}	0.84±0.20^{a}
Leucine (EA)	7.2±0.52^{b}	0.22±0.07^{a}
Phenyl alanine (EA)	3.8±0.22^{b}	1.98±0.02^{a}
Lysine (EA)	8.12±0.08^{b}	2.4±0.36^{a}
Tryptophan (EA)	1.86±0.26^{b}	0.6±0.13^{a}

Numbers within the same row followed by a different superscript letter were significantly different (P=0.05, DMRT)

Table 4 — Vitamin content of fish meals

Vitamin/kg	FFM	SFM
Vitamin A (IU)	54.65±3.75^{b}	12.23±0.87^{a}
Vitamin D(IU)	152.34±1.77^{b}	43.11±24.87^{a}
Vitamin E(IU)	35.7±1.75^{b}	9.0±0.5^{a}
Vitamin K(mg)	10.2±1.29^{b}	4.0±0.32^{a}
Ascorbic acid(mg)	54.0±1.0^{b}	25.0±1.67^{a}
CyanocobalaminB12(mg)	-	-
Biotin (mg)	-	-
Choline(mg)	43.76±1.10^{b}	8.4±0.52^{a}
Folic acid(mg)	In traces	-
Inositol (mg)	108.22±1.34^{b}	4.9±0.48^{a}
Niacin (mg)	14.0±1.0^{a}	-
Pantothenic acid (mg)	10.0±0.7^{b}	3.0±1.0^{a}
Pyridoxine (B_6)(mg)	In traces	-
Riboflavin (B_2)(mg)	7.0±1.0^{b}	1.0±0.0^{a}
Thiamine (B_1)(mg)	10.0±0.5^{b}	2.0±0.11^{a}

Numbers within the same row followed by a different superscript letter were significantly different (P=0.05, DMRT)

Table 5 — Microbiological quality analysis of fish meals

Microbial parameters	FFM	SFM
TPC(cfu/g)	2.3×10^{2}	2.0×10^{8}
TFC(cfu/g)	-	5.0×10^{3}
E.coli	Absent	Present
Staphylococci/g	0	1.4×10^{3}
Salmonella / 25g	Absent	Present
Vibrio / 25 g	Absent	Present
Pseudomonas/25g	Absent	Present
Clostridium/25g	Absent	Present
Listeria/25g	Absent	Present

FFM were below detectable level (BDL). Iron content was noted in FFM (48.36 µg/gm) and SFM (15.01 µg/gm). Copper content was a trace in FFM and SFM. The average manganese content was noted in the FFM (11.95 µg/gm) but it was below the detectable level in SFM. The average zinc content was high in FFM (32.36 µg/gm) and it was very low in SFM (8.4 µg/gm). The contents of micro elements such as selenium and iodine were 1.10 and 22.87 µg/gm, respectively in FFM but below the detectable level in SFM. The elements of cobalt, fluorine and chromium were found only in traces and BDL in both the fish meal samples. SFM had lesser amounts of heavy metals such as mercury, cadmium, arsenic, lead, nickel and chromium, but in FFM they were below the detectable levels. The overall estimate is that the mineral content of SFM is poorer than the FFM.

SPSS analysis was performed to differentiate the significant and non-significant variations in parameter values between the fish meals. Numbers within the same rows followed by a different superscript alphabet were significantly different (P=0.05, DMRT).

Microbiological Quality Analysis

The results of the microbiological quality of the fish meals are shown in Table 6. Higher bacterial count was observed in SFM (2.0×10^8) and lower count in FFM (2.3×10^2). Fungi were not found in FFM and SFM, and its cell density was 5.0×10^3 CFU/g with moisture content of 16.54 %. Absence of *E. coli* was noted in both SFM and FFM. The bacteria coagulase positive *Staphylococci* were absent in FFM, and present in SFM but the values were within the limit of 10^3 CFU/g recommended by ICMSF in Good Manufacturing Practice (GMP). The processing method significantly reduces the *Salmonella* in FFM though not in SFM. Thus, SFM alone had *Salmonella* contamination. Further, salt tolerant *Vibrio* sp. *w*as found in SFM. The specific spoilage microorganism *Pseudomonas* was only detected in SFM. The food safety hazards species of *Clostridium* showed positive results only in SFM. The disease listeriosis caused by *Listeria* species was observed in SFM. The study reveals that the SFM is poor in quality, showing rich microbial contamination, whereas fish meals prepared by other methods are acceptable.

Storage studies of fish meal

To study the effect of antioxidant in fish meal, part of fish meal was divided into two portions, one part was treated with 150 ppm ethoxyquin (EQ) antioxidant and other was untreated, and each portion was stored at ambient temperature for 6 months. The changes in moisture (%), TPC (CFU/g), TFC (CFU/g), TMA-N (mg N/100g), TVB-N (mg N/100g), FFA (% of oleic acid), PV (meq/kg of fat), and TBA (mg MDA/kg) were analyzed during the storage period of 6 months and the results are presented in Tables 7 and 8. Significant differences occurred during the period of storage between the antioxidant-treated and untreated fish meals. SFM with and without antioxidant initially showed higher moisture content (>10). Moisture content drastically increased in the untreated fish meal, but only slight changes were observed in the initial moisture level throughout the storage period in the treated fish meal. Antioxidant-treated fish meal showed limited microbial loads (up to10^5). Substantial increase of microbial load was

Table 6 — Storage studies of antioxidant treated and untreated fresh fish meals

Quality parameters	Storage period in months (Without antioxidant)							Storage period in months (With antioxidant)						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
Moisture (%)	5.8	6.47	6.9	7.07	7.49	7.92	10.11	5.8	5.8	6	6.1	6	6.1	6.13
TPC (cfu/g)	2.6×10^2	4.8×10^2	1.4×10^3	3.8×10^3	6.0×10^3	8.2×10^3	1.4×10^4	2.6×10^2	2.6×10^2	2.9×10^2	3.0×10^2	3.3×10^2	3.5×10^2	3.8×10^2
TFC (cfu/g)	-	-	-	-	1.0×10^2	1.4×10^2	3.2×10^3	-	-	-	-	-	-	-
TMA-N mg N/100g	1.22	1.5	2.3	5.07	9.05	16.2	17.1	1.22	1.22	1.29	1.3	1.31	1.31	1.55
TVB-N mg N/100g	19.9	26.6	34.7	36.1	41.3	42.9	46.7	19.9	19.95	19.95	20	20.12	20.15	20.22
FFA% of oleic acid	1.24	1.29	2.36	2.99	3.42	5	5.2	1.24	1.24	1.24	1.24	1.28	1.4	1.51
PV Meq/kg of fat	-	2.21	5.73	8.92	6.31	7.36	5.63	-	-	-	-	-	-	0.051
TBA(mg) MDA/kg of sample	0.034	0.4	0.59	1.3	2	3.98	4.5	0.034	0.034	0.079	0.085	0.099	1.01	1.03

Table 7 — Storage studies of antioxidant treated and untreated stale fish meals

Quality parameters	Storage period in months (Without antioxidant)							Storage period in months (With antioxidant)						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
Moisture (%)	16.54	17.8	17.9	18.8	23.6	25.9	28.6	16.54	16.3	16.3	15.5	16	16	16.5
TPC (cfu/g)	5.0×10^4	2.6×10^5	1.5×10^6	3.0×10^6	2.8×10^7	3.3×10^8	5.1×10^9	5.0×10^4	5.8×10^4	5.9×10^4	2.0×10^5	6.3×10^5	6.6×10^5	3.0×10^6
TFC (cfu/g)	5.0×10^3	1.9×10^4	7.4×10^4	8.3×10^4	1.1×10^5	1.5×10^5	3.5×10^6	5.0×10^3	5.0×10^3	6.8×10^3	7.0×10^3	2.2×10^4	2.5×10^4	2.0×10^5
TMA-N mg N/100g	35.56	38.2	41.6	46	48.7	52	57.5	35.56	36.47	38.12	38.5	39.14	41.11	43.6
TVB-N mg N/100g	93	96.2	105.6	118	121.4	125	128.1	93	93.91	93.96	93.98	94.04	94.05	94.76
FFA% of oleic acid	4.33	6.88	9.4	12.6	13.2	15.5	16	4.33	4.33	4.5	4.62	4.72	4.8	4.82
PV Meq/kg of fat	9.52	9.6	11.46	16.66	21.73	25.6	26	9.52	9.8	10.5	10.9	11	11.8	12.5
TBA(mg) MDA/kg of sample	3.5	3.6	3.9	4.6	6	6.3	7.4	3.5	3.7	3.9	4.3	4.3	4.4	4

noticed every month in the untreated fish meals. The growth of fungal colony was absent in 3-4 months initial storage of antioxidant untreated fish meal samples of FFM. On further storage, fungal growth was found to increase every month throughout the storage period, while in the antioxidant-treated samples fungal colonies were totally absent in both fish meals.

The acceptable shelf life of fish meal based on TMA-N value of antioxidant untreated samples was up to 5 months (16 mgN/100g) for FFM and (shelf life (35.56 mg N/100g) for SFM. Antioxidant treated fish meals have good shelf life except for SFM. TMA-N values within acceptable limit up to 6 months were gained for FFM. The antioxidant treated SFM fish meal had a limited volatile amine production. TVB-N significantly increased during the storage period and was observed to be high in the antioxidant untreated meals. TVB-N content of 6 months stored fish meals revealed that the FFM without antioxidant (46.70 mg/100g) was not close to the value found in FFM with antioxidant (20.22 mg/100g). The lipid qualities such as PV, TBA and FFA, presented in Tables 7 and 8, were also observed to have similar trend. Fish meal treated with the antioxidant showed limited lipid oxidation when compared to antioxidant untreated one. It is evident from the results that the antioxidant treated fish meals do not require refrigeration storage to get longer shelf life.

Discussion

Moisture content was found to be higher in SFM than in FFM. Ariyawansa[30] reported that the moisture content of fish meal depends on the condition of fish, nature of processing and drying. Kader *et al.*[31] reported that 11-12 % moisture content is the acceptable limit for fish meal. NRC[32] stated that the protein content of fish meal varies from 60.00-72.30 % depending on the type of fish and the method of preparation. The present study indicates that the lower protein content of SFM is because of the improper storage of fishes under refrigeration and this is in agreement with the observations of Ruiter[33]. In the present study, the lipid content was high in FFM (9.90 %) and low in SFM (4.83 %). The values of lipid contents in other notable studies are: 0.62 % in Kilka fish meal[34], 7.30 % in the Herring fish meal[35], 11.20 % in Mehhaden fish meal[36], 0.58 % in the Tilapia byproduct fish meal[5] and 10.28 % - 14.49 % in anchovy, red eye or Pilchard meals[37]. Hultin and Kelleher[38] reported that the change of lipid content in fish meal depends on the type and quality of fishes used. Fish meal has low fibre and is easy to digest, whereas plant materials have rich fibre content[39]. The fish meals of the present study have <5 % of fibre. Not much variation was observed in the ash contents of both the fish meals, but low level of ash content (11.48 %) was found in FFM. Similar values were reported for ash contents at 13.2 % in the Kilsa fish meal[40], and at 12.85 % in anchovy fish meal[15]. Ash content was low in the hygienically dried sample and the results are also in accordance with the earlier finding[41]. There was an increase in the NFE in the SFM. This increase might be due to the combination of the carbohydrates (glucose and glycoside units) and limitation of other nutrients and the present results are in agreement with the previous results[42].

The present study found a higher total cholesterol content (50 -70 mg/100g) in FFM and a lower value (19.6 mg/100g) in SFM. Criner and Feeley[43] reported

that fish muscles have 50-120 mg/100g cholesterol responsible for fatty acid metabolism and these are high density lipoprotein responsible for health of human beings. Rosselot *et al.*[13] reported that lipid oxidation reduced the cholesterol level in fish meal and it is in agreement with the SFM. Calorie content is indirect evidence of nutrient value of a particular product. In the present study, it was rich in FFM because it had high protein and lipid contents but in the other fish meal the calorie content was reduced due to the decrease in the nutrient content, and similar results have been reported earlier[44]. The quality indicator of fish meal such as protein solubility was high in SFM and nearly 40 % of soluble protein was observed but was absent in other fish meal. Gunnarasson[45] reported that the press liquid obtained from stale fish had more dissolved and suspended solids and proteins than that from fresh fish. The above findings are in agreement with the results of SFM. Hygienic FFM contained a low salt content of 0.9 %, but SFM had a higher amount of salt (3.9 %) and this value is well within the limit of <7 % specified for good quality fish meal[46]. Even without the addition of any salt, there is accumulation of the salts in fish body (Na+, K+ Cl+) due to the oozing out of water from the fish after the onset of rigor mortis[47]. The qualitative analysis reveals the absence of non-protein nitrogen in both fish meals with the evidence of absence of urea in the fish meals as an adulterant. Non protein substance has long been recognized as toxic to animals when it exceeds the limit of 6 %[48] with considerable evidence[49].

TVB-N (40 mg N/100g) and TMA-N (10-15 mg TMA-N/100 g) are generally regarded as the limit of acceptability for premium quality fish meal[50]. In the present study, total volatile amines exceeded the acceptable limit in SFM. Drying condition, temperature and freshness of the fish were responsible for the increase in TMA-N and TVB-N content in fish meal. FDA guidelines[51] reported that histamine present at the level of >5 mg/100g is probably toxic and >10mg is unsafe for consumption and results in histamine food poisoning called scombrotoxism[52]. In the present study, histamine did not exceed the limit of 10 mg/100g in FFM but SFM got higher value due to spoiled raw material. FFA value became unacceptable in SFM because it exceeded the acceptable limit of 5 % in fish meal, and high level of FFA is an indication of microbial spoilage activity[53]. Losada *et al.*[54] reported that accumulation of FFA has a detrimental effect on ATPase activity and protein solubility. Peroxide value is widely used as an indicator for the assessment of the degree of primary lipid oxidation. In this study, it was used to determine the quality of lipid. Peroxide value did not exceed the acceptable limit of 10 -20 meq per kg of fat[55].

Perez-Villareal and Howgate[56] reported that fatty acids in fish lipids react with molecular oxygen to produce the oxidation products of peroxides. In the present study, primary lipid oxidation was high in the stale fishes used for fish meal due to delayed processing of fishes or over-storage of raw fish, and lipid reaction with molecular oxygen increased the lipid deterioration, which is supported by earlier reports by Bragadottir *et al.*[57]. TBA is responsible for the rancid flavors and is used as an indicator for the assessment of the degree of secondary lipid oxidation, and TBA level did not exceed 3 mg MDA kg-1[58]. In the present study, no secondary oxidation products were observed either in stale fish meal (3.5 mg MDA/kg) or in fresh fish meal (<3 mg MDA/kg) and there was no onset of rancidity. These results are similar to those of fish meal for fish feed[59].

Of the twenty fatty acids common in aquatic animals fourteen were observed in both the fish meals[60], and the contents of most of the fatty acids were higher in fresh fish meal. The abundance of fatty acid composition is due to the freshness of raw material. Palmitic acid, myristic acid, lauric acid and capric acids, which are believed to have antimicrobial properties, were present in higher proportions in the fish meals[61]. Fishes normally have n-3 PUFA, due to variation of lipid quality and n-3 fatty acid content varied between the fish meals, and higher content was observed in the fresh fish meal.

There were eighteen amino acids detected in fish meals, whereas the number of EAA was around ten. This result indicates that the fish meals are good for human consumption also, but the amino acid levels, which depend on the quality of protein in fish meal, varied. The respective amino acid values for FFM and SFM are 69.75 and 32.95 %. The method of processing at low temperature enabled the fresh fishes to retain the availability of amino acid. With an increase in the processing temperature amino acids get denatured and their availability decreases. Olafsdottir *et al.*[62] reported fishmeal with high TVB-N value and with low values of both available amino acids and fatty acids. In the same way, in the present study, the availability of amino acids and fatty acids in the fish meal prepared from fresh oil sardine fishes

was higher due to the controlled conditions and utilization of fresh fishes, but in stale fish meal the low availability of amino acids and fatty acid is due to high TVB-N value. Fish meal prepared in the present study was considered to be a moderately rich source of water soluble vitamins especially niacin, choline, ascorbic acid, inositol, pantothenic acid and thiamine. According to Chilima[63] fat contributes to energy supply and assists in the proper absorption of the fat soluble vitamins A, D, E and K. Lipid peroxidation also affects the stability of vitamins in fish meal. This observation is in agreement with the results of the present study, as the peroxide value was rich in the stale fish meal. The break down products of lipid peroxidation can react with the epsilon amino group and reduce the biological availability of vitamins.

Calcium is the most abundant mineral in the South African fish meal with levels of 2.2 - 8.7 %[64]. In the present study, calcium was the most abundant mineral present in the fish meals, and was more abundant in FFM than SFM. Moghaddam[40] reported that in Kilsa meal the content of phosphorus was 74 µg/gm, sodium 63.23µg/gm, potassium 92 µg/gm, chlorine 0.64 µg/gm, magnesium 64.10 µg/gm, sulphur content 0.041 µg/gm, iron 129.3 µg/gm, copper 6.2 µg/gm, manganese 13.7 µg/gm, zinc 30.39 µg/gm, selenium 1.58 µg/gm and iodine content was 1.9 µg/gm. In the present study, when compared to previous results, with the exception of chlorine, phosphorus, sulphur and copper all the other minerals were found in larger amounts in prepared fish meals especially so in FFM and this finding coincides with an earlier report[65]. Fish meals should be free from toxic metals. Heavy metals of concern are mercury, cadmium, arsenic, lead, nickel and chromium, whose permissible limits are: arsenic (<0.0ppm), cadmium (<3.0 ppm), lead (<1.0 ppm), mercury (<1.0 ppm), nickel (80 ppm), chromium (12 ppm). In the present study, only stale fish meal had small amounts of some heavy metals but in fresh fish meal they were below the detectable levels.

Bacterial count of FFM only showed acceptable quality and SFM showed marginal and unacceptable quality and this variation may be due to processing method and quality of the fishes. Guillaume *et al.*[66] reported that poorly sterilized and preserved fish, loss of raw material freshness, and autolytic breakdown caused by enzymatic action are responsible for microbial population in fish meal, and the present study also observed similar result. Fungi cause direct losses in nutrient and quality of fish meal, and as a consequence fungus-infected fish have some poisonous mycotoxin which contaminates the finished feeds[67]. Mycotoxin was observed only in SFM, which indicates that the post-processing drying is responsible for the contamination. ICMSF reported that fish meal without pathogens such as *E. coli, Salmonella*, *Vibrio*, *Pseudomonas* and *Staphylococci* were nutritious to culture animal apart from maintaining the environment healthy[68]. In the present study all the pathogens were observed in SFM, which is due to the defective processing, processor and area of processing. *Vibrio* and *Listeria* were not detected in fish meals. Shikongo Nambabi *et al.*[69] reported that *Vibrio* present in seafood is a normal flora obtained from sea. Kabhahenda and Husken[70] reported that unhygienic processing, handling and contaminated environment are responsible for this contamination. Ashok Kumar[71] reported that the conditions of some landing sites are very poor in Tuticorin and domestic sewage is discharged right at the landing site of Tuticorin.

Ethoxyquin (ETOX) is a synthetic antioxidant (AOX) with a non-phenolic structure and has been the approved antioxidant used in fish meal production, and this was used in this study. Indian commercial manufactures also treat fish meal with 250 ppm antioxidant to extend the shelf life[72]. In the present study, ETOX (150 ppm/kg) was used in newly made fish meal packed on low density polythene bags. During storage period, the moisture content changed in both antioxidant treated and untreated fish meals. SFM both treated and untreated with antioxidant initially showed higher moisture content (>10). However, later the moisture content sharply increased in the untreated meal. In treated meal only a slight fluctuation was noted from the initial moisture level throughout the storage period. The fish meal treated with antioxidant showed less moisture content, which did not exceed the acceptable limit of 10 %. Moisture is an exact indicator of the susceptibility of a product to undergo microbial spoilage, and it also affects the stability and shelf life of the food product[73].

Of the 2 types of fish meals, the antioxidant-treated fish meal showed limited microbial load (up to10^5) but the load was high in SFM. Sharp increases of microbial load were noticed every month in all fish meals not treated with the antioxidant. ETOX though an antioxidant which inhibits oxidative degradation of the fish meal, does not inhibit growth of fungi[74].

Antioxidant untreated fish meal samples of FFM and SFM were free from fungal colony only up to 3-4 months, whereas in the antioxidant-treated samples fungal colonies were totally absent. The analysis of volatile amines such as TMA-N and TVB-N reveals that in the antioxidant untreated fish meal the amounts of volatile amines increased during the storage period. Total volatile nitrogen bases increased in fish tissues immediately after harvesting due to the effect of microorganisms as well as autolysis processes utilizing protein degradation products causing spoilage[75]. The result shows that the significant increase in the amounts of TVB-N during the storage period was higher in the antioxidant untreated meal. Survival of microbes was limited in antioxidant treated meal, as antioxidant reduces the oxidation in fish meal, and in the absence of suitable substrate microbes cannot grow. The formation of free fatty acids in stored fish meal both treated and untreated with antioxidant was assessed at the interval of 6 months. Opstvedt[76] reported that Capelin meal was more stable towards hydrolysis by the antioxidant addition (500 ppm ETOX) and demonstrated increased stability in the storage period. In the present study, antioxidant treated fresh and stale fish meals showed prolonged storage capacity up to 6 months. In contrast, the same fish meals even in good quality without antioxidant showed increase in the rancidity level in all the fish meals beyond three to four months. The initial quality of SFM shows high rancidity level and further storage changes were high in non-antioxidant samples. El-Lakany and March[77] reported that 125 ppm ETOX was enough to stabilize the meal lipids against oxidative changes and malonaldehyde formation.

Conclusion

This study demonstrates that the fish meal prepared by wet reduction from fresh fish and stored in frozen condition at -4 ^{o}C was good in many aspects, and this is confirmed by the quality indicators. But fishes iced after a delay or not iced at all or stored under refrigeration at 8 ^{o}C yield poor quality fish meals. The quantities and qualities of the biochemical constituents of fresh fish meal were better than those of stale fish meal. A fish meal with good quality has a longer shelf life of about 3 months and with the addition of antioxidant (ETOX), shelf life can be extended even up to 6 months. ETOX does not improve the quality of the fishmeal but maintains the freshness and quality during the storage period.

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