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Study on Changes of Protein and Lipid of Fish Protein Concentrate (FPC) Produced form Kilkas in VP and MAP Packages at Light and Darkness condition During Six Months

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***Abstract*— Fish protein concentrate (FPC) is a healthy, sustainable and high nutritive product that sanitizedly produced from fishes in which, protein and other nutrients are more concentrated than in fresh fishes. The aim of this research is to study on the sustainability of FPC produced from kilka in two VP (Vacuum Packaging) and MAP (Modified Atmosphere Packaging) packages in light and darkness condition during six months. According to our results protein rate was 91.2%, lipid: 0.5%, ash: 3.6%, moisture: 2.3%, TVN: 10 ml/100gr and peroxide: 5meq/kg. Amino acids includes; Alanin: 38.80, Argenine: 62.60, Aspartic: 73.20, Cysteine: 9.20, Glutamic: 122.40, Glysine: 34.00, Histidine: 29.40, Isolucien: 45.20, Lucien: 75.3, Lysine: 80.60, Metionine: 32.30, Phenylalanine: 69.80, Proline: 35.50, Serin: 31.70, Threonine: 43.40, Tyrosine: 30.60, Valine: 52.50 (mg/g) and fatty acids includes; Arachidic acid: 1.25, Oleic acid: 40.50, Stearic acid: 5.52, Palmitoleic acid: 27.95, Myristoleic acid: 0.28, Myristic acid: 2.94 (g/100g) were also determined. Amino acids and fatty acids were also determined. Lipid rate in FPC after 6 months in light conditions in VP package has changed from 0.50 to 0.43 %, in MAP package combined of 60% $C0_2$, 30 % N_2 and 10% O_2, was changed from 0.50 to 0.41 % which showed small decrease. In darkness condition in VP and MAP packages has changed from 0.50 to 0.49 and 0.47 % respectively that is not significant (P>0.05). It was also detected that light condition lead to more decrease in lipid content but that was not significant (P>0.05). Protein rate of FPC has changed from 91.2% to 85.40% during six months at light condition in VP Package and from 91.2% to 77.40% in MAP package that is significant (P<0.05). But at darkness condition protein rates were changed from 91.2% to 89.10% and 85.30% respectively in VP and MAP packages, these changes are significant (P<0.05) but more decrease in MAP package was detected again. Therefore, maintenance of fish protein concentrate is recommended under darkness condition.**

***Index Terms*—Fish protein concentrate, Kilka, Modified Atmosphere Packaging (MAP), Vacuum Packaging (VP).**

I. Introduction

Since variety of fishes as an animal protein source has a high nutritive value, consumption of them not only meets many nutritive requirements of body, but also is useful to improve human health so, many countries have tried to increase per capita consumption of this nutritive source [1].

Fish protein concentrate (FPC) is a healthy, sustainable and high nutritive product that sanitisedly produced from fishes in which, protein and other nutrients are more concentrated than in fresh fishes [2]. FPC was first proselyte widely in the late 1960s, as the most effective way to uproot global malnutrition [3].

Global Food and Agriculture Organization (FAO) has defined FPC as any sustainable product from fish for human consumption that has more protein than its raw materials, and divided in to three types:

- Type A: an odorless and flavorless powder which has maximum lipid content of 0.75%.
- Type B: a powder which has fish smell and flavor that has maximum lipid content of 3%.
- Type C: a kind of common flour which is produced in hygienic conditions.

FPC can play effective role in decrease protein deficiency in some crowded parts of world that suffered from malnutrition. Nutritive studies have shown exactly that adding FPC to diets has useful effect. Use this product is specially beneficial for babies growth and pregnant women [4].

FPC is a low cost animal protein with high quality, so can be used as a supplementary protein to increase nutritive value [5]. Considerable works were done to develop FPC producing method and use it in different foods, but unfortunately there is little information about sustainability of FPC during stocking in different environmental conditions [6].

In this study kilka was used to produce FPC. Kilkas are so

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sensitive, tiny and slender; therefore always some parts of their body loss their proper quality to produce different products that can be directly used by human. Kilka is one of the most important economic fishes in Caspian Sea. It belongs to family Clupeidae [7]. It is a native species of Caspian Sea, Black Sea, Azouph Sea and found in all parts of Caspian Sea specially in costal line. Three species are identified from kilka in Caspian Sea: *Clupeonella engrauliformis* (Anchovi), *C. grimmi* (Large eyed Kilka), *and C. cultriventris* (Common Kilka) (Figure. I).

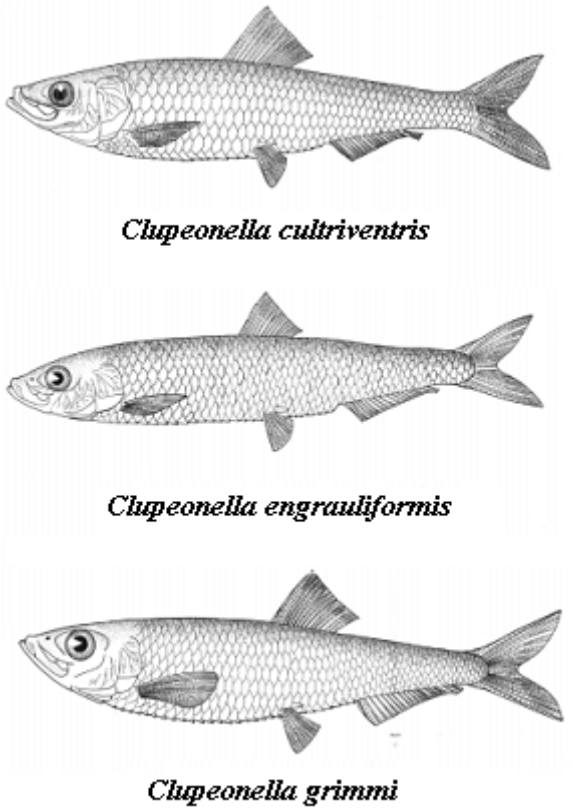

Figure I: Three *Clupeonella* species of Caspian Sea.

Clupeonella engrauliformis and *C. grimmi* are endemic to the Caspian Sea, while *C. cultriventris* is found in the Caspian and Black seas. *Clupeonella* species have a dark blue, blue-green or light green back, silvery flanks and a silvery or silvery-white belly. Fins are mostly hyaline (transparent or nearly so) although the dorsal fin may have a central dark stripe and the caudal fin dark base. Life span of *Clupeonella* species is up to eight years with maturity at 1-2 years. Fishes aged 2-4 years dominate catches. Populational and yearly differences in growth are common depending on variations in productivity. Southern populations grow faster than northern ones and females faster than males. *C. cultriventris*, together with the sturgeons, comprises 82.1 percent of the fish biomass in the Caspian Sea. *Clupeonella* species take mostly zooplankton, especially copepods but also mysids, fish fry, cladocerans, and *Balanus* and clam larvae. The vertical and seasonal migrations of *C. engrauliformis* mirror that of its principal food item, the copepod *Eurytemora grimmi*, which can comprise over 70 percent of its diet. The three *Clupeonella* species share the available habitat and its foods, *cultriventris* in shallow coastal waters, *engrauliformis* in the upper layers of the open sea and *grimmi* in deeper waters of the open sea. *Clupeonella* species are important foods for the Caspian seal, sturgeons (as much as 60 percent of their diet), *Alosa* species, pikeperches (*Stizostedion* species), Caspian salmon (*Salmo trutta caspius*) and inconnu (*Stenodus leucichthys*). Predators consume 590 million kilograms of kilkas each year making these fish a very important element in the life of the Caspian Sea [8].

The *kilka* or *Clupeonella* catch in the Iranian Caspian Sea reached 51,000 tons in 1994 from none 10 years previously. A ceiling of 100,000 tons has been suggested from a resource of 800,000 tons. The catch is taken by the Industrial Fishing Company and fishing cooperatives using artificial lights as attractants, deep conical nets, and air lifting. A small portion of the catch is used in a high value form as frozen whole consumer packs, smoked, salted and canned in sauce and the rest is used as fish meal for poultry and in aquaculture. *C. cultriventris* comprises only 1.35 percent and *C. grimmi* only 6.84 percent of the Iranian catch of kilkas which is dominated by *C. engrauliformis* at 91.8 percent. This is attributed to the larger spawning and foraging range of the latter species [8].

The aim of this study is to investigate the sustainability of FPC produced from kilka in two VP and MAP packages at light and darkness condition during six months.

II. Materials and Methods

To produce FPC from kilka, a method given by FAO was used [4]. First, fishes were captured from Caspian sea, Bandar Anzali, then transferred to national processing researches center with ice and sea water.

Fishes were washed with hygienic water, then, their heads, tails and viscera were removed. After that, they were transferred to deboner device to remove their bones, skin and fins from meat. Pure meat was transferred to isopropanole in the proportion 2:1 (alcohol : fish) at environment temperature (25.8°C) for 50 min, after this period, primary press was done and prepared press cake transferred to second phase of concentrate production. In this phase press cake was placed in isopropanole in the proportion 2:1 (alcohol: fish), at 75°C for 90 min in a benmari. Then it was pressed again and prepared press cake was placed in solvent in the same proportion and due to enter third phase of extraction was hold in benmari at 75°C for 70 min. It was pressed again and transferred to a dryer at 125°C. This product was grinded and passed through 100µm filter. At First, Chemical factors were determined to evaluate qualitative specifications of FPC produced from kilka fishes. Then this product was packaged in 100gr units in Vacuum Packaging and Modified Atmosphere Packaging (MAP) condition (60% CO_2, 30% N_2 and 10% O_2), and investigated at light and darkness condition during six months to evaluate the protein and lipid rates.

Protein rate was determined by kajeldal [9] and lipid rate by Bligh and Dyer [10], moisture rate by oven method [11], ash rate by electrical stove [12], peroxide indicator (PV) by Lee method [13], evasive nitrogen rate (TVN) by cacro kajeldal[11], amino acids rate by HPLC device, (model: Younglin) , fatty acids rate by GC device (model:Hewlett-Packard 6890).

Statistic analyses: The data were subjected to analysis of variance (one-way ANOVA), using LSD range test. The scheme of the FPC production is shown in Fig. II.

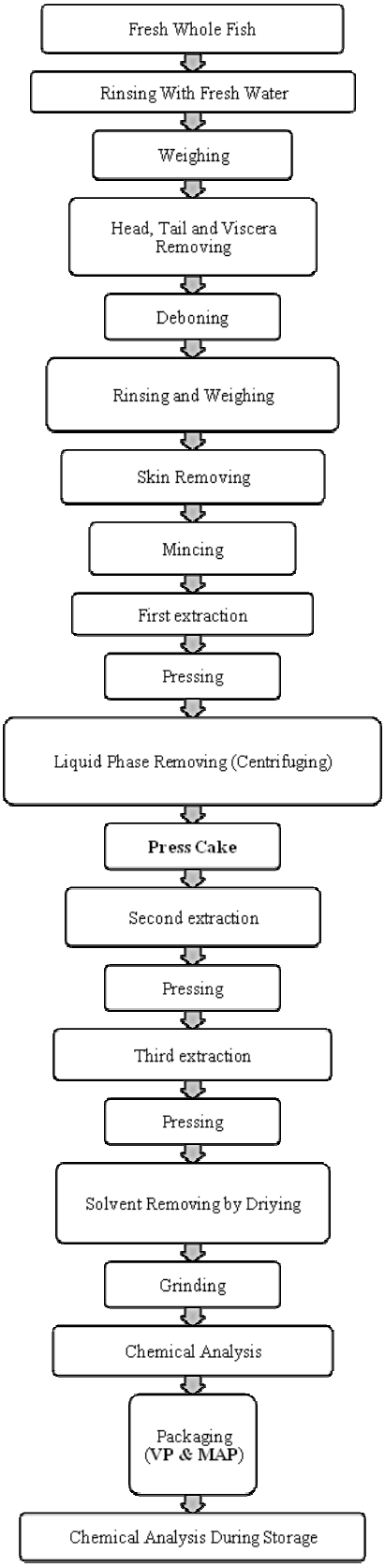

Figure II. Flow chart of fish protein concentrate (FPC) production

III. Results

Chemical compositions of fish protein concentrate produced from kilka are presented in table I; the rates of amino acids from FPC produced from Caspian Sea kilka were resented in table II and the rates of fatty acids in FPC produced from Caspian Sea kilka are present in table III.

TABLE I: COMPOSITIONS OF FISH PROTEIN CONCENTRATE PRODUCED FROM KILKA.

FPC	Ave
Moisture (%)	2.3
Ash (%)	3.6
POV (Meq/kg)	5.00
TVN (Mg/100g)	10.00
Lipid (%)	0.50
Protein (%)	91.2

TABLE II: THE RATES OF AMINO ACIDS FROM FPC PRODUCED FROM CASPIAN SEA KILKA.

Amino acid	Value (mg/g)
Aspartic	73.20
Glutamic	122.40
Serin	31.70
Glysine	34.00
Histidine	29.40
Argenine	62.60
Threonine	43.40
Alanin	38.80
Lysine	80.60
Proline	35.50
Tyrosine	30.60
Valine	52.50
Metionine	32.30
Cysteine	9.20
Isolucien	45.20
Lucien	75.30

Phenylalanine	69.80

TABLE III: THE RATES OF FATTY ACIDS IN FPC PRODUCED FROM CASPIAN SEA KILKA

Fatty acid	g/100g
Arachidic acid	1.25
Oleic acid	40.50
Stearic acid	5.52
Palmitoleic acid	0.48
Palmitic acid	27.95
Myristoleic acid	0.28
Myristic acid	2.94

A. Results of FPC lipid content produced from kilka in light and darkness condition

The percent of lipid in FPC preserved in light and darkness condition in VP packages are presented in table IV. Lipid rate has not been changed significantly in light conditions and preservation time.

TABLE IV: LIPID CHANGES IN VACUUM PACKAGES AT LIGHT AND DARKNESS CONDITION (%).

Light (%)	Darkness (%)	Li / Months
0.48	0.50	1
0.48	0.51	2
0.47	0.48	3
0.46	0.49	4
0.45	0.49	5
0.43	0.49	6

The lipid percent of FPC preserved at light and darkness condition and MAP packages are presented in table V. Small decrease is observed in lipid percent due to storage in light conditions and stocking time , but these changes were not significant (P>0.05).

TABLE V: LIPID CHANGES IN MAP PACKAGES AT LIGHT AND DARKNESS CONDITION (%).

Light (%)	darkness (%)	Li / Months
0.46	0.50	1
0.47	0.48	2
0.45	0.49	3
0.43	0.48	4
0.44	0.47	5
0.41	0.47	6

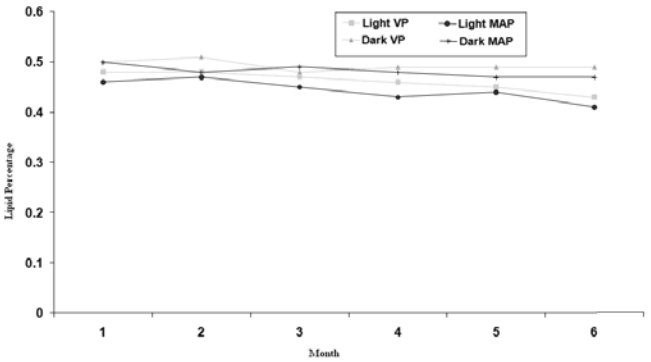

Figure III: Comparison of lipid changes in VP and MAP in light and darkness environments during 6 months.

B. Results of FPC protein content produced from kilka in light and darkness condition

The data are presented in tables VI and VII, show that protein content of FPC preserved at light and darkness condition, is decrease with storage in light conditions and stocking duration, it have been also observed more decrease in protein content in MAP packages in comparison with VP packages.

TABLE VI: COMPARISON OF PROTEIN CHANGES IN VACUUM PACKAGE AT LIGHT AND DARKNESS CONDITION DURING 6 MONTHS.

light (%)	darkness (%)	Pr / Months
89.50	91.10	1
87.40	90.00	2
87.10	89.60	3
86.30	89.40	4
86.20	88.90	5
85.40	89.10	6

TABLE VII: COMPARISON OF PROTEIN CHANGES IN MAP PACKAGE AT LIGHT AND DARKNESS CONDITION DURING 6 MONTHS.

light (%)	Darkness (%)	Pr / Months
88.90	89.20	1
87.10	89.10	2
84.80	88.70	3
83.27	87.80	4
80.10	86.20	5
77.40	85.30	6

Figures III and IV show the trend of lipid and protein changes of vacuum (VP) and modified atmosphere packed (MAP) FPC, stored in light and dark conditions during six months.

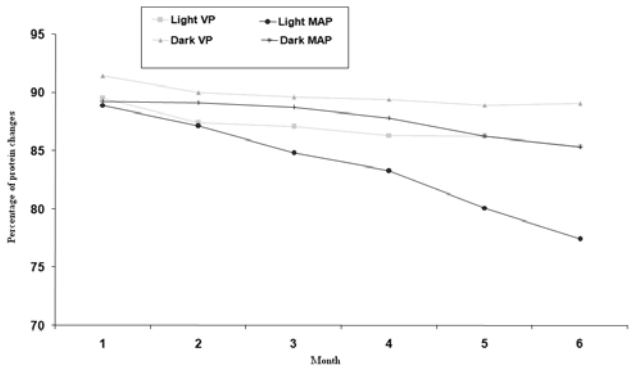

Figure IV: Comparison of protein changes in VP and MAP in light and darkness environments during 6 months.

IV. Discussion

As shown in table I, protein and lipid rates are respectively 91.2% and 0.5%. According to FDA guideline, FPC can be used in human diets as a nutritive supplementary, if addition to have high hygienic quality its protein content is more than 75% and its lipid content is less than 0.5%. Although FAO has accepted maximum lipid rate of 0.75%. According to FAO and FDA guidelines, our produced FPC is classified as type A, and can be used as a rich protein source.

In a research was done by Azhdary and his cooperators [14] on production of FPC from silver carp, 81% protein and 0.37% lipid were reported, so FPC produced from kilka has higher protein rate because of different reasons. FPCs depend on the fishes types, have different chemical and physical specifications, it can be affect on final chemical compositions of product [15].

About 20% minerals and 78% protein were determined in FPC produced from menhaden fish due to have more bones [2], instead, FPC produced from hake contains 13% minerals and 85% protein [5]. Lipid content is also different in any kind of fishes, but during press and extraction process by lipid solvent, it decreased to less than 1%, although in this case, FPC produced from different fishes didn't show so many differences.

Lipid content in FPC in table I is 0.5%, according to tables IV and V, after six months at light conditions, lipid content in VP package was changed to 0.43% so it didn't show significant variation and in MAP package, it was changed to 0.41% that showed small decrease, it is because of O_2 presence in MAP package and oxidation of lipids. It is also detected that light conditions leads to induce and accelerate oxidation. In the study was done by Chen and Gong to investigate lipid oxidation during preservation of raw red claw crayfish tail meat in VP and MAP (80% CO, 10% O2 and 10% N2) packages during 14 days at 2°C, have been detected that lipid oxidation in VP packages was occurred lower than in MAP packages [16].

In the study was done by Cluskey and his cooperators to investigate the oxidation changes on milk powder in VP and MAP packages, it was detected that the lowest amounts of lipid and cholesterol oxidation were occurred in VP packages [17]. In another research was done by Medwadowski and his cooperators [18], extractable amounts of lipid from FPC with 0.5% lipid after 6 months at 37°C and very significantly in 50°C were decreased. Also, in these samples the rates of neutralized lipids, free fatty acids, C20:5 and C22:6 poly unsaturated fatty acids were decreased.

According to tables VI and VII, protein rate of FPC (91.2%), after 6 months at light conditions, was changed to 85.40% and 77.40% respectively in VP and MAP packages. It is due to the fact that O_2 presence and aerobic bacterial reactions, but at darkness conditions, protein content in VP and MAP packages were respectively changed from 91.2% to 89.10% and 85.30%. These changes are very small but higher in MAP package again these changes were significant ($P<0.05$). In the study was done by Chen and Gong [16] to investigate lipid oxidation during preservation of raw red claw crayfish tail meat in VP and MAP (80% CO, 10% O_2 and 10% N_2) packages during 14 days at 2°C, have been detected that protein denaturation in MAP packages was much more than in VP packages.

In comparison with our results, an electriphorogram that investees the effects of wet heating at 100°C during 0, 1, 3, 6, 12 and 24 hours on flour of gram, bean and faba bean, it has been shown that high changes in decrease of thickness and color of protein bands in relation with heating time enhancement was occurred. Inactivation of trypsin inhibitors arose from intensive effects of heating and trypsin on hydrolysis of sample proteins [19]. All of these researches suggests that lipid and protein changes in VP package are much less and their quality is better preserved, and preservation of foods away from light leads to maintain nutrients quality.

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