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The Effects of Different Storage Temperatures and Durations on Peroxide Values of Fish Feed Ingredients[#]

Aysun Kop[1,a,*], Kutsal Gamsız[1,b], Ali Yıldırım Korkut[1,c], Hülya Saygı[1,d]

[1]*Department of Aquaculture, Fisheries Faculty, Ege University, 35100 Bornova/Izmir, Turkey*
[*]*Corresponding author*

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ABSTRACT

The growth of fish in intensive fish farming is carried out completely by the feeds supplied from externally. Different ingredients are used in feed production. The nutritional content of these ingredients is checked when purchased or brought to the factory. These ingredients are then stored until feed production. Storage duration and storage temperatures directly affect the freshness criteria of feed ingredient materials. Especially when high-energy ingredients with high levels of fat are stored in poor storage conditions, the fats in the ingredients are oxidized, therefore the peroxide number increases and the ingredient becomes bitter. Oxidation not only destroys the lipids in fish feeds but also vitamins. Slow growth, poor feed evaluation, color darkening, lethargy and deaths have been reported in fish fed with diets that are oxidized and inadequate in vitamin E. In this study, oxidation levels of fish feed ingredients were determined due to different storage conditions. Generally, the number of peroxides increased due to the increase in storage time and temperature, depending on the type of raw materials and oil content.

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Farklı Depolama Sıcaklıkları ve Sürelerinin Balık Yem Hammaddelerinin Peroksit Değerleri Üzerine Etkileri

MAKALE BİLGİSİ

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ÖZ

Entansif balık yetiştiriciliğinde balığın büyümesi tamamen dışarıdan verilen yemler sayesinde gerçekleşmektedir. Yem yapımında birçok farklı hammadde kullanılmaktadır. Bu hammaddelerin besinsel içerikleri satın alındığında ya da fabrikalara getirildiğinde kontrol edilmektedir. Daha sonra bu hammaddeler yem yapımına kadar depolanmaktadır. Depolamanın süresi ve depolama sıcaklıkları doğrudan yem hammaddelerinin tazelik kriterlerini etkilemektedir. Özellikle yağ düzeyi fazla olan yüksek enerjili yemlerin kötü depolama koşullarında uzun süre bekletilmesi durumunda yem içindeki yağlar oksitlenmekte, buna bağlı olarak peroksit sayısı yükselmekte ve yemlerde acılaşma meydana gelmektedir. Okside olmuş yemlerde sadece yağ bozulmamakta aynı zamanda yemde bulunan vitaminler de bozulmaktadır. Okside olmuş ve E vitamini bakımından yetersiz yemlerle beslenen balıklarda, büyümede yavaşlama, kötü yem değerlendirme, renkte koyulaşma, letharji ve uzun süreli beslemelerde ise balık ölümlerinin görüldüğü bildirilmektedir. Bu çalışmada, farklı depolama koşullarına bağlı olarak balık yemi hammaddelerindeki oksidasyon dereceleri belirlenmiştir. Genel olarak, Peroksit sayıları hammaddelerin türüne ve yağ oranına bağlı olarak, saklama süresinin artmasına ve sıcaklığın artmasına bağlı olarak artış göstermiştir.

[a] *aysun.kop@ege.edu.tr* *https://orcid.org/0000-0003-1724-5672*
[b] *kutsal.gamsiz@ege.edu.tr* *https://orcid.org/0000-0003-3277-9488*
[c] *ali.korkut@ege.edu.tr* *https://orcid.org/0000-0002-1096-5725*
[d] *hulya.saygi@ege.edu.tr* *https://orcid.org/0000-0002-3408-6709*

Introduction

The sustainability of aquaculture depends on the continuity of quality feed production. It is very important to take into account the biological-physiological characteristics and nutrient requirements of the fish. Besides, feeds should be good quality, affordable, healthy and do not harm the environment, (De Silva and Anderson, 1995; Karabulut et al., 2000; Yiğit and Yiğit, 2003; Demir, 2008; Bostock, 2011).

The researchers stated that feed costs constitute 30-70% of the production cost in carnivorous fish farming (Metailler, 1986; Korkut and Yıldırım 2003; Akyurt, 2004; Bostock, 2011; Özerdem et al., 2013). Therefore, the quality of the feed should be monitored in fish production. For this purpose, it is always important to know the raw materials which constitute the first stage of feed production and to prepare ration accordingly.

Many different raw materials are used in feed production. The nutritional content of these raw materials is checked when purchased or brought to the factory. These raw materials are then stored until feed production. The duration of storage and storage temperatures directly affect the freshness criteria of feed raw materials. Especially when high-energy raw materials with high-fat levels are kept in bad storage conditions for a long time, the fats in the feed are oxidized, and the number of peroxides increases and rancidity occurs in the feed. Peroxide is a term used for hydroperoxide compounds that are eventually released by oxidation reactions and these compounds are Powerful catalysts and accelerate oxidation reactions. Oxidized fatty acids have a toxic effect. As a result of oxidation, oils, vitamins, pigments are at risk. Hydroperoxides reduce the availability of vitamins (A, D, E, K). Besides, oxidized fatty acids react with lysine to reduce the use of amino acids. It is not desirable to have more than 1% moisture in fish oil. In the case of excess, the oxidation is accelerated (Korkut et al., 2007). In oxidized feed, not only the fat is spoiled but also the vitamins in the feed are spoiled. Oxidized and vitamin E inadequate feeds in fish feed, slow growth, Poor feed evaluation, color darkening, lethargy, long-term feeding is reported to be seen in fish deaths.

Storage of feeds containing fish oil at high temperature results in an increase in both. Fish fed with oxidized feeds reported poor growth, poor feed efficiency, dark body coloring, anemia, lethargy and increased mortality due to rancid food in long-term feeds (Tacon, 1992).

In this study, oxidation levels of fish feed raw materials were determined due to different storage conditions.

Material and Methods

Raw materials were obtained from companies that produce fish feed in Turkey and companies that sell to the fish feed sector. Raw materials used in the trial; Anchovy meal, Peruvian fish meal, Soybean meal and Poultry meal, Black Sea fish oil (produced from anchovy + Sprat mixture), Sprat oil, Anchovy oil, Salmon Fish oil, Aquaculture by-products oil, Salmon by-products oil. All samples were stored at room temperature (20°C), refrigerator (4°C) and at 30°C to represent higher temperatures. Storage times are 60 days for oil, 30 days for solid ingredients. Fish oils are divided into three groups according to the storage temperature, each group being arranged to be 3 repeated. A different method has been applied in the sampling and analysis of solid raw materials compared to liquid raw materials. Solid raw materials were kept in environments representing different storage conditions, and the oil values were separated and PO values were measured at the end of the storage period. Oils in solid raw materials were separated using the Bligh-Dyer method (1959) to determine the degree of oxidation. Peroxide values of oils were measured by titration of liberated iodine with standardized sodium thiosulphate solution according to the AOAC official method 965.33 (AOAC 1990). According to AOAC 965.33 titration method; 10 g of chloroform, 15 ml of acetic acid and 1 ml of potassium iodide were placed in 1 g of oil and shaken vigorously. The samples were kept in a dark place for 5 minutes. Then 75 ml of purified water and 1 ml of starch were added to the samples. The samples were titrated with 0.01 M sodium thiosulfate until the yellow color has almost disappeared. Calculation;

$$\text{Number of Peroxides (PO)} = (V \times N) / P$$

V = Amount of the used sodium thiosulfate,
N = normality of 0.01 N sodium thiosulfate,
P = the amount of sample.

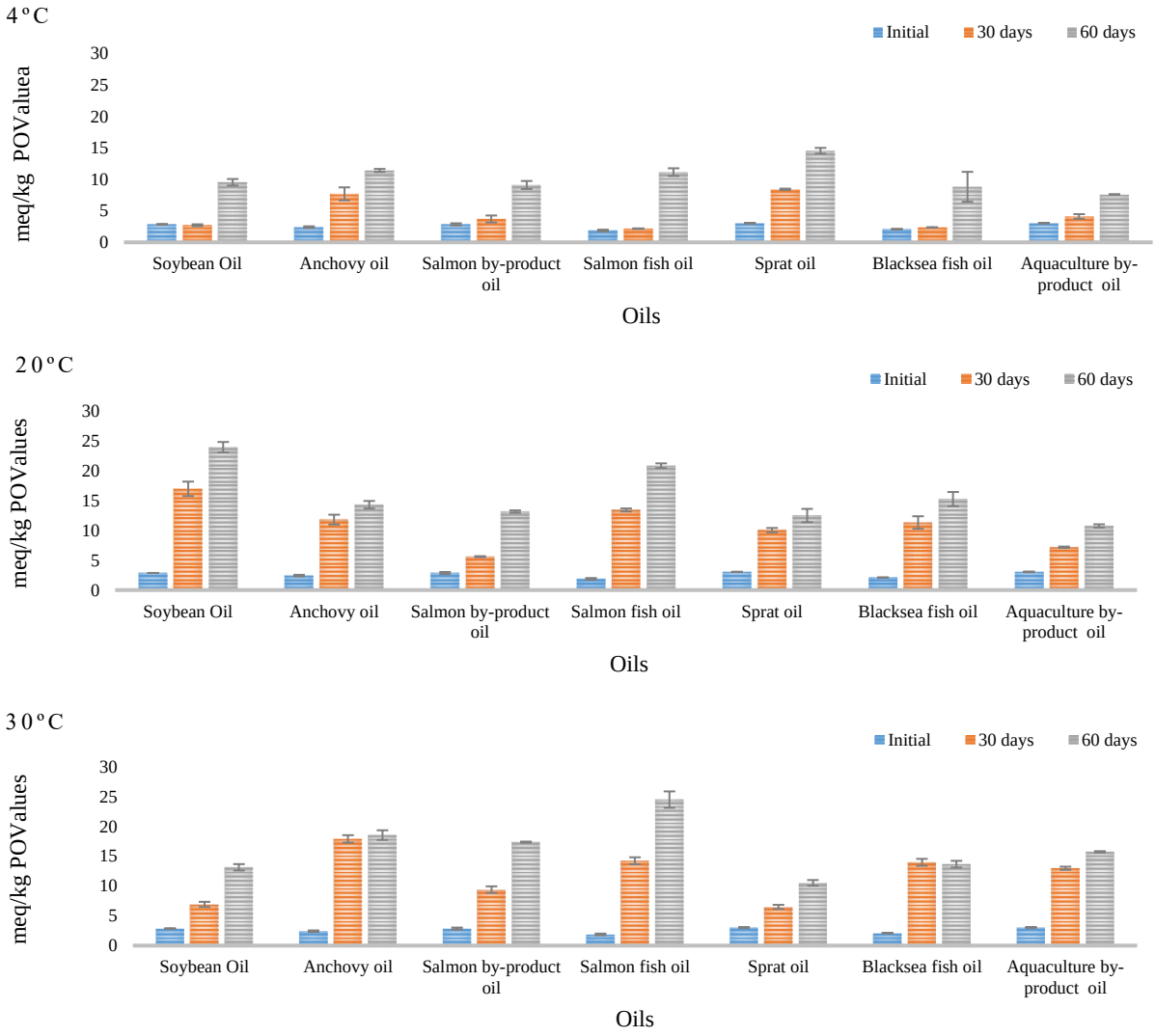
Significant differences ($P<0.05$) between mean values were determined by the analysis of variance with the assistance of SPSS25.

Results

Peroxide Value Results of Oils

According to the results of the analysis, peroxide values obtained from various oils are given in Graphic 1.

The initial PO values of soybean oil for all experimental groups were 2.85 ± 0.01 meq/kg. Samples with an ambient temperature of 30°C reached 6,91±0,43 meq/kg PO at the end of the first month and 13,12±0,81 meq/kg PO at the end of the 2nd month. When the results were evaluated according to months, it was found to be statistically different from each other ($P<0.05$). At the end of the study, the PO values reached by these samples were outside the acceptable limit (10 meq/kg). The PO values of the samples with an ambient temperature of 20°C at the end of the 1st and 2nd months are much higher than the samples kept at the other temperature. Accordingly, significant differences were observed when the results were evaluated according to months ($P<0.05$). The difference between the samples with the ambient temperature of 20°C compared to the samples kept at other temperatures in the same months was also statistically significant ($P<0.05$). The PO values reached by these samples at the end of the study were above the maximum limit (10 meq/kg). In the refrigerated samples, the value reached at the end of the first month was close to the initial value and no statistically significant difference was found ($P>0.05$). However, the results of the second month increased to make a significant difference ($P<0.05$).



Graphic 1 Peroxide Values of Oil Samples Based on Storage Temperature

The initial value of anchovy oil samples was determined as 2.41±0.13 meq/kg. Accordingly, at the end of the first month, the PO value of the samples kept at 30°C was measured as 17.78 meq/kg. This value is above the acceptable limit (10 meq/kg). In samples held at 20°C, this value was 11.78 ± 0.82 meq/kg and at 4°C, it was 6.58±1.03. In the second month, although the values showed an increase, there was no statistical difference between the results of the first month and the second months of the samples held at 30 and 20°C (P>0.05). However, the values obtained depending on the storage time for the samples at 4°C differ statistically (P<0.05), (Figure 5). In general, at the end of the second month, PO values reached only at 20 and 4°C are close to acceptable limits.

The initial PO value for salmon by-product oil was 4.22±0.17 meq/kg. The PO values of the samples held at 30°C reached 9.39±0.55 meq/kg PO at the end of the first month and 17.35± 0.08 meq/kg PO at the end of the 2nd month. Although there is a certain increase in the PO values of the samples kept at other ambient temperatures compared to months, these are not very significant increases. All oxidation values are acceptable in Salmon by-product oil.

Salmon fish oil has the lowest starting value (1.87±0.14 meq/kg) among the experimental groups. At 20°C and 30°C it reached very high values at the end of the first month. At the end of the second month, there is still an increase in values and above the maximum limit. Only the PO values of the samples at 4°C are within acceptable values.

The initial value of sprat oil was found to be 3.03±0.04 meq/kg. In the first and second month analysis of the samples, an unexpected result was obtained in PO analyses and it was found that the samples at 30°C reached the lowest PO value compared to the others. The PO values obtained in all groups were within acceptable limits.

The initial PO values of the Black Sea fish oil were measured as 2.09±0.05 meq/kg. Samples held at 30°C reached 13.96±0.58 meq/kg PO at the end of the first month and remained unchanged at 13.64±0.56 meq/kg at the end of the second month (P> 0.05). Samples stocked at 20°C reached 11.29±1.05 meq/kg PO at the end of the first month and 15.21±1.18 meq/kg at the end of the second month. This value is higher than the samples kept at 30°C. The first month results of the samples kept at 4°C showed a slight increase compared to the initial value and reached the PO value of 2.37±0.01. The difference between the initial value and the results of the first month was

statistically significant (P <0.05). At the end of the second month, this value increased to 8.82±0.45 meq/kg.

The initial PO value of the aquaculture by-products oil was 3.04±0.06 meq/kg. PO values of the samples were measured as a 12.96±0.26 meq/kg at 30°C, 7.15±0.12 meq/kg at 20°C, 4.11±0.37 meq/kg at 4°C at the end of the first month. The values reached for each group were found to be significantly different from initial values (P <0.05). At the end of the second month, the samples at 30°C increased to 15.70±0.12 meq/kg, while the samples at 20°C showed a value similar to the values of the first month. However, there was a statistically significant difference (P <0.05).

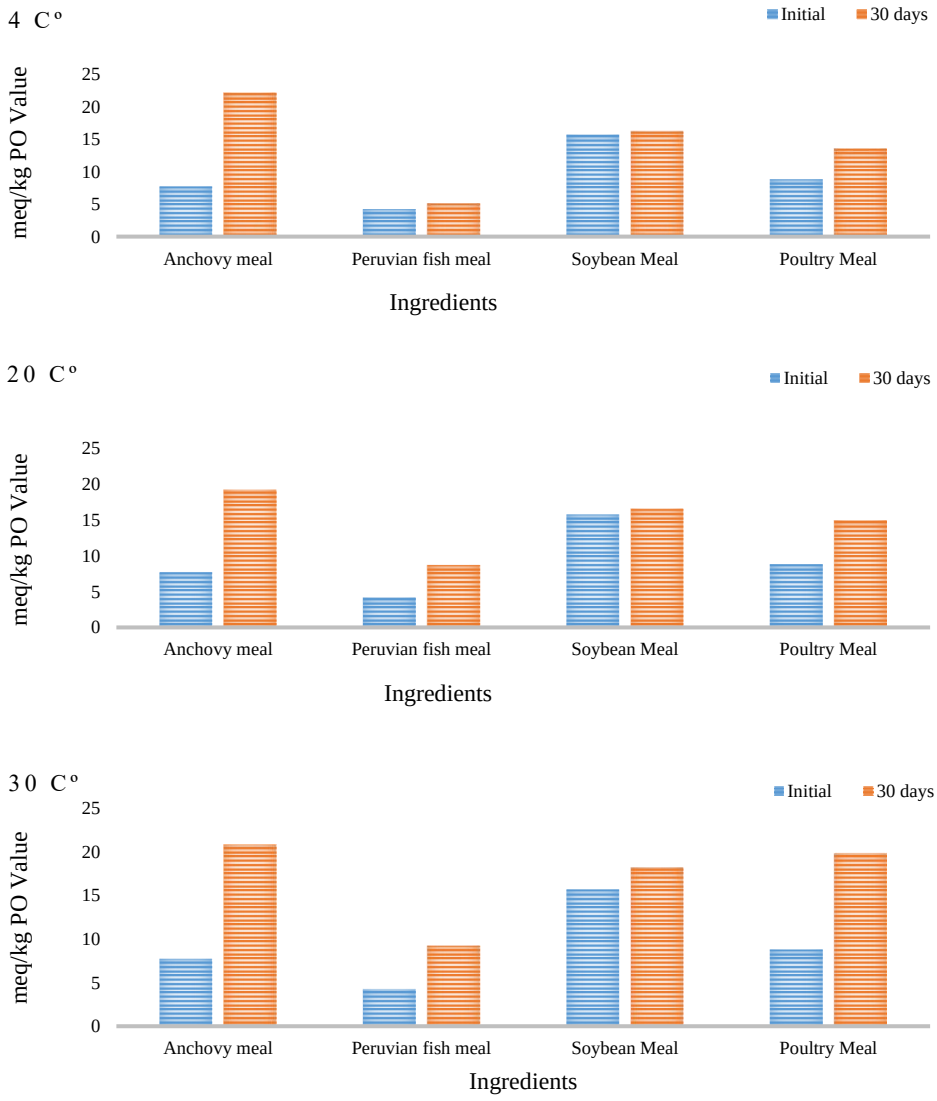
Peroxide Value Results of Solid Ingredients

According to the results of the analysis, peroxide values obtained from various solid ingredients are given in Graphic 2.

The initial PO value of the anchovy meal was 7.7 meq/kg. PO values of anchovy meal are at the maximum limit at the end of the first month. The obtained values indicate that the samples reached the highest PO value in the refrigerator environment. This is followed by samples kept at 30°C and 20°C, respectively. At the end of the first month, there was no significant difference between groups (P> 0.05). The values are around a maximum of 20 meq/kg PO values.

The initial PO value of the oil extracted from the Peruvian fish meal was measured as 4.21 meq/kg. PO values reached 9.24 ± 0.94 at 30°C, 8.66 ± 0.34 at 20°C and 7.80 ± 0.73 meq/kg at 4°C. At the end of the first month, no significant difference was found between the storage temperatures (P> 0.05).

The initial PO value of the oil extracted from the soybean meal was determined to be 15.7 meq/kg. PO values at the end of the first month were measured as 18.23±0.49 at 30°C, 16.48±0.78 at 20°C and 16.19±1.68 meq/kg at 4°C. Although there seems to be a decrease at 20°C and 4°C compared to an initial value, there is no significant difference between the initial and first month values (P> 0.05). However, when the results were analyzed statistically according to the storage temperature of 30°C, a significant difference was found (P <0.05). The oil obtained from the soybean meal was initially oxidized and remained constant. The numerical difference seen is thought to be due to sampling.



Graphic 2 Peroxide Values of Solid Ingredients Based on Storage Temperature

The initial PO value of the oil extracted from the poultry meal was 8.8 meq/kg. At the end of the first month, PO values reached 19.82 ± 30.4°C, 0.49°C at 14.86 ± 0.78°C and 13.54 ± 0.76 meq/kg at 4°C. At the end of the first month, a significant difference was found ($P < 0.05$).

Salmon fish oil initially having the lowest peroxide value (1.87 meq/kg) among the fish oils stored at high temperature (30°C) reached the highest peroxide value (24.47 meq/kg) at the end of the experiment. The highest peroxide value at 30°C was found in Salmon by-product oil after Salmon whole body fish oil. There was no difference between the 1st and 2nd months of Anchovy oil and Blacksea fish oil at 30°C. At the end of the 1st month, there was no difference between the storage results of Sprat oil at 20 and 30°C. There is an increase in the peroxide values of all oils at the end of 1 and 2 months at storage at 20°C. At the end of the first month, peroxide values in Salmon oil, Salmon by-products and Blacksea fish oils kept in the refrigerator did not show much change compared to the initial peroxide values. At the end of the second month, peroxide values of all oils increased. However, these values are acceptable.

Discussion

The initial PO values of the lipid samples are below 5 meq/kg which should be in the quality fish oil (Young, 1985). At the end of the first month, the PO values were again below 5 meq/kg in most of the lipid samples kept at 4°C. Only anchovy and sprat oil increased above these values. All fat PO values showed a certain increase at the end of the second month. However, these values are still below the acceptance values of 15 meq/kg.

PO values of samples stored at 20°C temperature increased at the end of the first month. The PO values of some samples were found quite high. These are Soybean oil (16.91±1.21), Salmon oil (13.39±0.58), anchovy oil (11.78±0.82), Black Sea origin fish oil (11.29±1.05), Sprat oil (10.02±0.36). Although the PO values of the oils obtained from Salmon by-product oil and Aquaculture by-products also increased, it was observed that the PO values in the samples kept at 4°C were as low. The inclusion of antioxidant substances in oils produced from wastes can cause this. All samples except soybean oil are within acceptable PO value. At the end of the second month, the PO values of all samples reached the acceptable value limit, while soybean oil and Salmon oil exceeded this limit.

PO values of samples with storage temperatures of 30°C increased at the end of the first month. Salmon, Salmon by-product and Sprat oils values increased compared to baseline values but were relatively lower than other fat samples. Anchovy oil exceeded the acceptable limit with its PO value of 17.87±0.62 meq/kg at the end of the first month. The remaining samples are within this limit. The Salmon oil PO value in the second month is far beyond the maximum limit. Anchovy and Salmon by-product oil also reached values very close to this limit.

Contrary to expectations, the values of soybean oil at 20°C were higher than those of 30°C. Similarly, PO values of sprat oil at 4°C for the first month were higher than those of 30°C in the second month and 20°C and 30°C in the second month. Salmon fish oil, which had the lowest peroxide value (1.87 meq/kg) among the fish oils stored at high temperature (30°C) at the beginning of the experiment, reached the highest peroxide value (24.47 meq/kg) at the end of the experiment.

The number of peroxides in oil increased with increasing temperature and storage period. When the results are evaluated according to the storage period, it is determined that at 20°C and 30°C at the end of the 2nd month, many fish oils have acceptable levels of peroxide in the vicinity and some of them are in a state of rancidity. However, peroxide values of some oils stocked at 20 and 30 degrees did not change. The reason for this is that the oxygen value does not change. In general, the oxidation rate increases with temperature. As the temperature increases, partial pressure changes of the oxygen have less effect on the reaction rate of oxidation. Because oxygen becomes less soluble in lipids and water. Attention should be paid to ambient temperature, time and light effect in the storage of various oils. Besides, minimum contact should be provided of these oils with oxygen.

When the PO value changes of the lipids obtained from solid ingredients are examined according to the temperature and storage time, it is seen that the oil obtained from anchovy meal has reached the highest PO value after one month at 4°C. At this temperature, the most stable product was a Peruvian fish meal. It is thought that this is due to the antioxidant application in the production stages of fish meal. It has been reported that the main factor limiting the oxidation of lipids in the presence of natural chemical antioxidants with different chemical structures that can affect the antioxidant activity and oxidation rate of fats (Nagy et al., 2016). The lipid obtained from soybean meal also maintained the highest PO value at the end of the first month. It is considered that this example has reached the maximum PO value in transactions applied (lipid extraction) to determine the initial PO value and remains at the end of the first month. PO values of the samples kept at 20°C are similar to those stored at 4°C. The highest PO value was measured in oil samples obtained from anchovy meal and the lowest was measured in oil samples obtained from Peruvian fish flour. PO values measured at the end of the first month are slightly higher than those of samples held at other temperatures.

When the results of all the samples are compared, it is understood that the oxidation in the solid ingredients occurs during the lipids extraction process from these materials.

Chemical processes that take place under the influence of factors such as oxygen, light and high temperature determine the condition of the oil. In this regard, the storage conditions of the raw materials are extremely important. Oxidative changes in oils depend on storage conditions such as temperature, oxygen and light, and the type of packaging (Kachel-Jakubowska et al., 2018). Free radical chain reactions start in oils into contact with oxygen (Jomova and Valko, 2013), and light can initiate oxidation with single oxygen characterized by an extremely high reactivity (Tańska and Rotkiewicz, 2003). It is reported that the oxidative stability of oils is strongly dependent on the chemical composition and reactions between unsaturated fatty acids and reactive oxygen species (Kachel-Jakubowska et al., 2018). The types and amounts of compounds that are oxidation products in the oil depend mainly on the fatty acid composition and storage

conditions (Kachel-Jakubowska et al., 2018). According to Bautista et al., (1992) and Ramezandeh et al., (1999), oxidative and hydrolytic spoilage start at high stocking temperature and it is the reason of the feed quality losses. Van den Bergh et al. (1990) and Ruiz et al., (2000) showed that oils are naturally unstable when exposed to high temperatures above 30°C. Under these conditions, lipids are hydrolyzed to release ketonic acids that undergo auto-oxidation along with the degeneration of free radical products (Hamilton, 1989). According to NRC (1981), lipid and vitamins break down with peroxidation of the lipid component in the feed stored at ambient temperature for more than 90 days (three months). The rancidity caused by lipid oxidation is the most significant change in spoilage during storage. Feed components that contain highly unsaturated lipids, such as fish feeds, are susceptible to oxidation (Pezzuto and Park, 2002; Sidhuraju and Backer, 2003). Chan (1987) reported that Polyunsaturated lipids can be autoxidation rapidly at ambient or low temperatures. Esterbauer et al., (1986), reported that fish oil PO values increased depending on the duration of contact with the air because the oil contains sensitive Polyunsaturated fatty acids.

The fish oil samples tested contained different levels of hydroperoxide. The results may depend on many factors such as fish processing and fish oil production, storage, added antioxidants and the presence of metals and light (Turner et al., 2006). Similarly, Halvorsen and Blomhoff (2011) studied the PO values of various fish oils sold by different companies in Norway and found that they contain different levels of hydroperoxide and alkenyl. They attributed these differences to the factors mentioned above.

In general, oils become bitter when the PO value is about 10 meq/kg. The PO value of a fresh and refined product should be less than 1 meq/kg (Gunstone, 1996). However, more specifically, different PO value upper limits for fish oils have been proposed. For example, Turner et al., (2006) recommended 2 meq/kg for a maximum level of Boran et al., (2006) suggests a value of 8 meq / mL, while Kolanowski (2010) suggests a value of 10 meq/kg.

Considering the recommended upper limit of 8 or 10 meq/kg PV, only a few products included in this study have reached this upper limit. However, if the lowest limit is accepted as a 2 meq/kg PV, most products exceed this limit. Similarly, Turner et al. (2006) analyzed six different commercial fish oils and found POand anisidine values in the range of 3.2-5.5 meq/kg and 9-20, respectively. They concluded that most fish oils on the market contain more hydroperoxide and secondary oxidation products than recommended.

Halvorsen and Blomhoff (2011) stated that fish oils contain high amounts of lipid oxidation products because they contain high amounts of unsaturated and easily oxidizable fatty acids such as EPA and DHA compared to vegetable oils.

In this study, the effects of storage temperature and duration on the quality of oils obtained from various fish species and feed raw materials were investigated. Storage temperature has been found to have significant effects on the storage stability of fish oil. Fish oil samples stored at 4°C had a shelf life of almost twice longer than samples stored at 30°C. In addition, it has been found that the oxidative and hydrolytic stability varies greatly depending on the fish species used to produce oil.

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