THE OXIDATION OF FISH OIL DURING EXTRACTION PROCESS AND STORAGE

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ABSTRACT

Fish oil has high nutritional value, but easily undergoes oxidation to form many undesirable products. The aim of our study was to evaluate the effect of fish oil extraction methods on the extraction yield and the oxidation state of the product. Besides, the effect of natural and synthetic antioxidants on the quality of fish oil during extraction and storage were also examined. Extraction methods selected in this study included: NMKL-No.131 method, Soxhlet method and mechanical methods with and without addition of ginger and tea extract. The oxidation of extracted fish oil was evaluated by peroxide value (PV). The results have shown that the method NMKL-No.131 had provided highest extraction yield, but the oil had been the most oxidized. Meanwhile, the mechanical method provided lower extraction yield, but the quality of the product was much more better, met the requirements of commercial fish oil product. The addition of ginger and tea extract to fish suspension before extraction lowered the peroxide value of extracted fish oil significantly. The presence of ginger and tea extract in fish oil also retarded the oxidation, this effect was as high as the effect of the popular synthetic antioxidant BHT.

1. INTRODUCTION

It has been well documented that fish oil possesses many health benefits. Fish oil provides source of important ω-3 fatty acids whose effects on human have been widely studied. The results from many works have shown that ω-3 fatty acids lower the level of triglyceride in blood, antiarthritic, improve memory and ability to concentrate. ω-3-fatty acids also prevent cancer, improve vision, lower blood pressure and more other medical benefits. That explains why fish oil is an important product in world trade.

The fatty acid composition of fish oil is completely different from those of land animals. Fish oil contains large amount of unsaturated fatty acids (84%), so it exists in liquid state at ambient temperature. In fish oil the content of C_{14} –C_{16} rather low, C_{18}, C_{20} are most dominant, C_{18} is much more than C_{22}-C_{26}. Fish oil extracted from fresh fish is generally colorless or yellowish, but some oils from certain fish may have red colour due to the presence of carotenes. During processing and storage, fish oil becomes dark brown, even black [1,4].

In fish oil manufacturing, quality assurance plays an key role, especially the prevention of oxidation is very important, because this product is very vulnerable to oxidation due to high content of polyunsaturated acids.

The aim of our current study was to examine the effect of extraction method choice on the oxidation of obtained fish oil, besides we also assessed the possibility to apply natural antioxidants from ginger and green tea to improve the quality of the product during processing and storage.

2. MATERIALS AND METHODS

2.1. Materials

The fish used for extraction were herring and catfish, which were bought from market in Da Nang City.

The materials used for extraction of natural antioxidants were ginger (from Da Nang) and green tea (from Thai Nguyen)

Chemicals used in our experiments were from China.

2.2. Methods

2.2.1. Extraction of fish oil

Extraction of fish oil in our study, we used three different extraction methods:

a) Extraction according to Method NMKL-No.131 (Nordisk metodikkommitté för livsmedel) [5]

Samples were treated with hydrochloric acid 8 M and then were added with ethanol 96%. The lipid freed was extracted with mixture of diethyl ether and petroleum ether. The solvent was evaporated and the remain lipid was weighed [3].

b) Extraction according to Soxhlet Method with petroleum ether [9].

c) Extraction by means of heating and mechanical pressing [7].

Principle: The material is heated in water to 60^{o}C and hold for 18 min in order to free lipid in liquid state; Liquid lipid is insoluble in water and rises on the surface; Remove water to obtain fish oil (oil phase 1); Transfer the fish to the hydraulic press and under mechanical force the extract is removed from the fish meal. Remove water to obtain fish oil (oil phase 2). Combine oil phase 1 and oil phase 2.

2.2.2. Extraction of natural antioxidants

a) Extraction of antioxidants from ginger: Ginger is cleaned, peeled, sliced and dried. After that, it is ground to powder. Weigh 5g of ginger powder to the 100 ml beaker, add 50 ml ethanol 96^{o}. Leave the slurry for extraction in dark for 48h. After that, the extract is centrifuged at speed of 3500 rpm. The supernatant is used as antioxidant extract.

b) Extraction antioxidants from green tea: Green tea is ground. Weigh 3 g of the tea into two necked round-bottom flask 250 ml and add 60 ml of distilled water. Fix a condenser and a thermometer. The extraction is carried out at 70^{o}C for 20 min. After cooling the extract is centrifuged. The supernatant with Bx = 2,01 is used as tea antioxidant extract.

2.2.3. Determination of peroxide value [9]: According to AOAC 965.33

Peroxides formed during lipid oxidation free I_2 from KI in an acid medium. The amount of freed I_2 is determined by titration with standard solution $Na_2S_2O_3$.

3. RESULTS AND DISCUSSION

Generally, there are two ways to extract lipid from animal or plant materials: the mechanical process (using pressing machines) and extraction methods (using appropriate

solvents that can dissolve lipid). Besides, there is another way that utilizes heat to melt lipid and then cools down to separate lipid from aqueous environment.

In our study, we focused on how to obtain the high extraction yield and the best quality of lipid after the extraction process. The quality of lipid is generally characterized by acid value, iodine value, peroxide value…We chose peroxide value to monitor the quality of extraction products, because this is the characteristic that easily excess the limit of allowance for commercially using purposes.

3.1. Evaluating the extraction yield and the quality of fish oil extracted according to Method NMKL-No.131

The mixture of diethyl ether and petroleum ether was used in this method. To evaluate the extraction yield of the Method NMKL-No.131, we used the "Fish paste" standard (from Norway) with the known lipid content 18.0%.

Lipid from fish paste was extracted by the Method NMKL-No.131. The extraction yield was calculated as percentage of the lipid content obtained from this method in comparison with the reported lipid content of the standard (18.0%). Experiment was carried out in triplicates and the results were shown in Table 3.1.

Table 3.1. The extraction yields of lipid oil extracted by the Method NMKL-No.131

Fish lot	1	2	3	Mean
Extraction yields (%)	99,15	99,06	99,18	99,13

This table shows that the ability to extract lipid of this method was very high (over 99%), so this would be an ideal method for completely remove lipid from fish. We then used it as the standard method for determining lipid content of fish samples in the following experiments.

The evaluation of the quality of fish oil extracted by the Method NMKL-No.131 was carried out as follows. Fish were bought from the market in 3 different days and classified as 3 different lots, every lot contained fish of only one day. Extraction was carried out in triplicate for every fish lot and peroxide values of extracted fish oils were analyzed. The results presented in Table 3.2 were the means value of extraction triplicates.

Table 3.2. The peroxide values (meq/kg) of fish oil samples extracted by the Method NMKL-No.131

Fish species	Herring			Catfish		
Fish lot	1	2	3	1	2	3
Peroxide value (meq/kg)	60,53	62,02	62,63	20,08	22,17	21,08

According to Chol Su Pak [2], the peroxide value allowed in commercial fish oil should be less than 8 meq/kg, meanwhile the Vietnamese Standard TCVN 6044:2007 allows the maximum limit of peroxide value in plant oil product equal to 10 meq/kg.

The results from table 3.2 shows that the peroxide values of obtained fish oil samples were very high, exceeded the limit of allowance for commercial use (8 meq/kg). The peroxide values

of herring samples were higher than those of catfish samples. This is easily understandable because herring is kind of fish which is very rich in the content of polyunsaturated fatty acids like DHA. During extraction process, these acids were exposed to the air, high temperature for long time, so they were deeply oxidized. Meanwhile, the catfish was much rich in the content of saturated fatty acids, so they were less oxidized.

From these experiments, we suggest that the method of NMKL-No.131 is suitable mainly for determination of lipid in fish.

3.2. Evaluating the extraction yield and the quality of fish oil extracted according to Soxhlet Method

In this Soxhlet method, the petroleum ether was used as solvent for extraction fish oil.

To evaluate the yield of fish oil, we used 2 methods to extract fish oil from the herring and catfish samples of 3 fish lots: the Method NMKL-No.131 and the Soxhlet method. The extraction yields were calculated as percentage of lipid obtained by the Soxhlet method in comparison with the lipid extracted by the Method of NMKL-No.131.

In addition, the peroxide values of oil extracted by the Soxhlet method were also analyzed in order to evaluate the quality of the products.

The results of measurement of extraction yields and peroxide values are presented in Table 3.3.

Table 3.3. The peroxide value (meq/kg) and the extraction yield of lipid in the Soxhlet method

Fish species	**Herring**			**Catfish**		
Fish lot	1	2	3	1	2	3
Extraction yield (%)	85,45	84,47	86,72	83,13	80,21	84,32
Peroxide value (meq/kg)	20,62	22,19	19,72	10,98	12,17	11,99

The results in Table 3.3 indicates that the Soxhlet method also provided high extraction yield (from 80.2% to 86.7%), but it was still not as effective as the Method NMKL-No.131. The loss of some lipid content in Soxhlet method occurred because samples had not been treated with HCl and ethanol as done in the Method NMKL-No.131, so a portion of complex lipid linked to protein might have not been freed for complete extraction.

Further more, Table 3.3 also shows that the peroxide values of fish oil samples extracted by the Soxhlet method were much less than those by the Method NMKL-No.131 (ranged from 10 -12 meq/kg for catfish and 20 - 22 meq/kg for herring). However, these values were still not low enough to satisfy the requirement for use commercially (PV < 8 meq/kg). The extraction procedure according to Soxhlet method included some steps such as drying samples, solvent evaporation, in which the samples of fish were exposed to light and were heated at high temperature, so the lipid oxidation could have been accelerated. That explains for the high peroxide values of the oils obtained.

3.3. Extraction fish oil by heating and mechanical pressing

After examining two previous methods of fish oil extraction, we noticed that the oil from herring was much more sensitive to oxidation than the oil from catfish, so we decided to choose

herring as the only material to use in study of the third extraction method. Fish oil from herring was extracted according to the scheme in Figure 3.1.

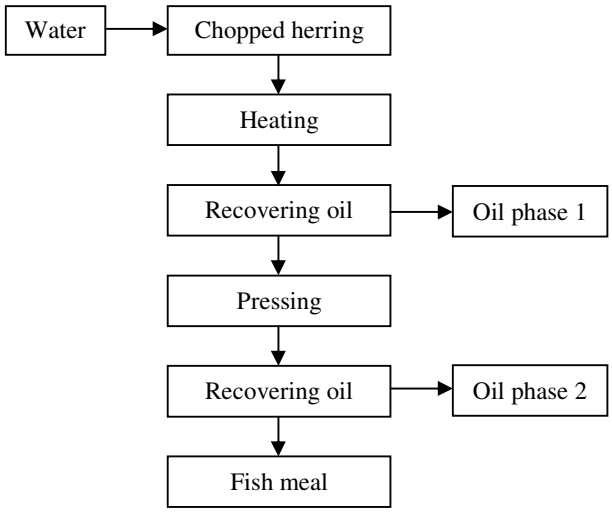

Figure 3.1. The scheme of fish oil extraction by heating and mechanical pressing

According to this scheme, fish were chopped, added with water. The ratio between fish and water was 1:2.5 (w/w). The suspension was then heated to 60 ^{o}C and held for 18 min. The liquid content was transferred into the extraction flask and the bottom water layer was removed. We obtained the first oil phase. The remain fish was pressed by an hydraulic press to give the liquid extract that was transfer to the extraction flask. Again we removed the water layer to recover the second oil phase.

After dissolving two oil phase together, the product was weighted and compared with the lipid content determined by the Method NMKL-No.131. Besides, the peroxide value of the oil product was also examined.

The results were shown in Table 3.4

Table 3.4. The peroxide values (meq/kg) and extraction yield (%) in the mechanical method using heating and pressing

Fish lot	1	2	3
Extraction yield (%)	82,10	83,20	80,07
Peroxide value (meq/kg)	4,32	5,07	3,52

According to Table 3.4, the extraction yield of this method range from 80.07% - 83,2%, this ratio was rather good, nearly equal to the extraction yield of the Soxhlet method. However, in contrast with the previous two methods, the peroxide value of the oils obtained by this method was much more lower. The peroxide values of all extracted fish oil samples were less than 8

meq/kg, among them the best sample had the peroxide value of 3,52 meq/kg. The difference of peroxide values between fish lots could be explained by the different initial quality of fish obtained from different days.

The lower peroxide values of the oils extracted by this method than other studied methods could be explained as follows. In this method, fish were submerged in water during heating and time of the whole extraction process was much shorter than the previous methods, so the fish oil was less exposed to oxygen and heat and then less oxidized. In addition, this method like Soxhlet method does not have steps of treating fish with HCl and ethanol, so the complex lipids linked to protein may not be completely freed for extraction. That is why the extraction yield of this method was lower than the yield of the Method NMKL-No.131.

3.4. The effect of ginger and tea extract on the oxidation of fish oil extracted by heating and pressing

The objective of this study was to examine if we could lower the peroxide value of fish oil extracted by the studied method of heating and pressing by means of ginger and tea extract.

In this study, we used herring bought from 2 different days and distinguished as Lot 1 and Lot 2. Ginger and tea extract were prepared according to 2.2.2. Fish oil were extracted according to the previous method of heating and pressing with some modification. Before heating, the fish-water suspension was added with ginger extract or tea extract (10% w/w on base of fish weight).

The results of measuring peroxide values of obtained oils were presented in Table 3.5.

Table 3.5. The effect of ginger and tea extract on the peroxide value of extracted fish oil

PV (meq/kg)	Control	Ginger	Tea
Lot 1	5.21	4.78	4.08
Lot 2	6.04	5.66	5.29

From the results shown on Table 3.5, it is noticed that all ginger and tea extract lowered the peroxide value of fish oil samples obtained, the tea extract had greater effect than ginger extract. With Lot 1, the peroxide values of samples with addition of tea and ginger extract were respectively 21.6% and 8.3% lower than the peroxide value of control sample. Meanwhile with Lot 2, the peroxide values of samples added with tea and ginger decreased 12.4% and 6.3% respectively.

The antioxidant activity of ginger and tea have been reported in some papers [3, 6, 8]. The antioxidant activity of ginger is supposed to be linked to the presence of gingeroloids and diarryl heptanoids, while the polyphenols named catechins are shown to be responsible for the antioxidant effect of green tea [8]. It was the presence of these active compounds that inhibited the oxidation of fish oil during the extraction process, resulting in lowered peroxide values. Generally, antioxidants are more effective at the initial stage of oxidation when the amount of peroxides is still low. When lipid is already significantly oxidized, the amount of antioxidants becomes insufficient to inhibit all radicals, so the antioxidant effect is not very clear. That explains why the antioxidant effect in case of Lot 2 was not as good as in case of Lot 1.

This preliminary study on the effect of ginger and tea extract on the oxidation of fish oil during extraction has shown the potential of using these extracts in fish oil production. Further more, in order to produce fish oil with good quality, it is essential that fish must be fresh, still not oxidized much.

3.5. The effect of natural and synthetic antioxidants on the oxidation of fish oil during storage

Fish oil is not stable during long time storage. The self-life of fish oil could be extended if the oil is added with antioxidants. Vitamin E is safe natural antioxidant, but its antioxidant activity is not as good as the popular synthetic antioxidants like BHT, BHT… and the price of vitamin is also higher. Meanwhile, the use of synthetic antioxidants are currently restricted because of their potential carcinogenic activity that has been reported in some works [8].

The aim of this study was to evaluate the antioxidant capacity of ginger and tea extract in fish oil in order to evaluate the possibility to use them for replacing the above mentioned synthetic antioxidants.

Eight flasks with 5 g of herring oil were prepared. Each pair of samples was added with 10% of ginger extract (w/w; base on ginger powder and weight of fish oil), 10% of tea extract (w/w; base on dried tea and weight of fish oil) and BHT 200 ppm. The 4. pair of samples without any addition was used as the control.

All prepared beakers were closed and stored in dark at room temperature. Every 2 days, samples were taken for measuring peroxide value. The results were presented in Figure 3.2.

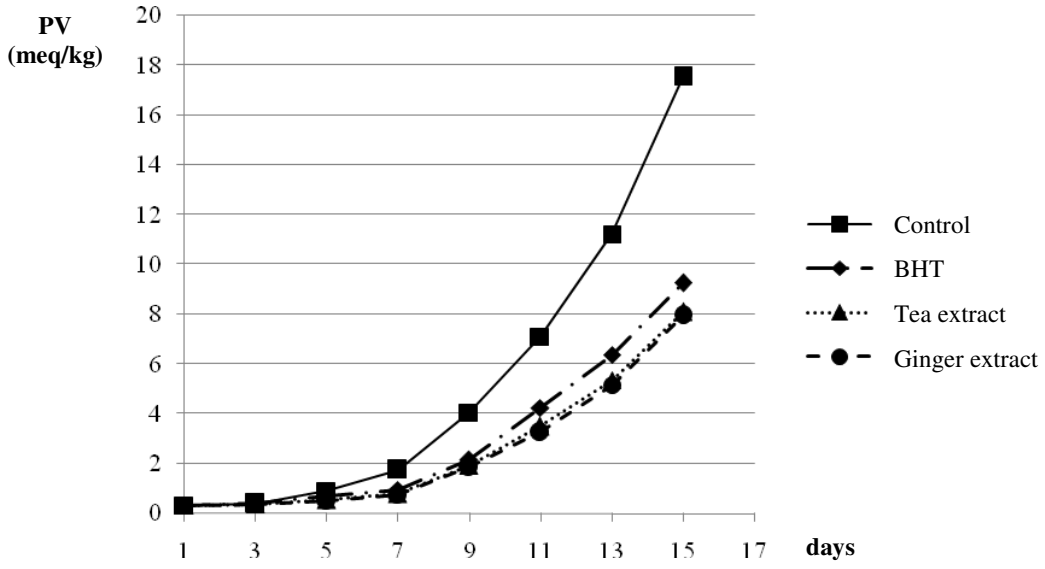

Figure 3.2. The change of peroxide value (meq/kg) of fish oil during storage

From Figure 3.2 we could notice the difference in peroxide value of the control and the remain samples. The peroxide values did not change much during the first 5 days, they increased very slowly. After the 7 day, peroxide value of the control started to increase rapidly, meanwhile

the peroxide values in other samples were nearly unchanged. Just after the 9.day the peroxide values of samples with addition of antioxidant started to increase faster. This indicated that BHT and the ginger and tea extract inhibited the formation of free radicals in the initial stage of oxidation of fish oil, therefore extended the initial period to 9 days in comparison with 7 days of the oxidation of the control.

The difference of peroxide value between samples with addition of ginger extract, tea extract and BHT was not significantly different, what suggested that the antioxidant activity of ginger and tea extract on fish oil were equivalent to the activity of the synthetic BHT. This result has shown the successful application of ginger and tea extract for protection of fish oil against oxidation during storage. Because of their natural origin, these antioxidants are expected to be safe for human.

4. CONCLUSION

Vietnam has a rather well developed fishery industry, the materials used for fish oil production are abundant, what is the condition for efficient development of fish oil production industry. To ensure the quality of fish oil, the matter of protecting product from oxidation is very important. Using antioxidants is an effective way, however the use of synthetic antioxidants which are highly effective and cheap, is currently restricted because of their doubtful safety. Therefore, research in the world has been focusing on how to replace synthetic antioxidants with safe natural antioxidants.

Our study has shown that among the studied methods for extraction of fish oil, the mechanical method utilizing heating and pressing had been proved to provide oil with the best quality, while the extraction yield had been also sufficiently high (>80%). The addition of extracts from tea and ginger to fish before extraction had significant effect on lowering the peroxide value of extracted oil. These extracts also extended the self-life of the fish oil during storage.

The mechanical extraction procedure of fish oil with heating and pressing need to be optimized in order to improve the extraction yield as well as the quality of the product in respect not only to peroxide value, but also to other quality characteristic affected by addition of extracts like flavour…

REFERENCE

1. Hoàng Đức Như - Dinh dưỡng và cuộc sống, Chất thông minh có trong cá Basa , NXB Y học, Hội Dinh dưỡng TP. HCM, 2003 .
2. Chol Su Pak - Stability and quality of fish oil during typical domestic application, Wonsan University of Fisheries, Kangwon Province, D.P.R. of KOREA, 1989.
3. Dang Minh Nhat, Takacsova Maria, Nguyen Dac Vinh, Kristinanova Kitti, Antioxidant activity of essential oils from various spices, Nahrung **45** (1) (2001) 64-66.
4. Hamilton R. J., Rice R. D. - Fish oil technology, nutrition and marketing, SCI oil and fats group, 1995.

5. Kasutoshi Miwa, Low Su Ji - Laboratory manual on analytical methods and procedures for fish and fish products, Department Southeast Asian, Fisheries Development Center, Singapore, 1992.
6. Kikuzaki H. and Nakatani N. - Antioxidant Effects of some Ginger Constituents, J. Food Sci. **58** (1993) 1404-1410.
7. Oistein Hostmark - Process for the extraction of fat/oil from fat fish, UK Patent Application, 1990
8. Pokorny Jan et al. - Antioxidants in Food-Practical Application, Woodhead Publishing Limited, Cambridge, London, England, 2001.
9. Pribela Alexander - Analyza potravin, Vydavatelstvo STU, Bratislava, Slovakia, 1989.

TÓM TẮT

NGHIÊN CỨU SỰ OXY HÓA CỦA DẦU CÁ TRONG QUÁ TRÌNH CHIẾT VÀ BẢO QUẢN

Dầu cá có giá trị dinh dưỡng cao, nhưng lại rất dễ bị oxy hóa, tạo những sản phẩm không mong muốn. Mục đích nghiên cứu của chúng tôi là đánh giá ảnh hưởng của các phương pháp chiết đến tỉ lệ thu hồi và sự oxy hóa của sản phẩm thu được.Tác động của các chiết xuất gừng, chè và chất chống oxy hóa tổng hợp đến chất lượng dầu cá khi bảo quản cũng được đánh giá. Các phương pháp chiết được chọn trong nghiên cứu gồm có: phương pháp NMKL-No.131, phương pháp Soxhlet và phương pháp đun nóng kết hợp ép cơ học có bổ sung và không có bổ sung chiết xuất gừng và chè xanh. Quá trình oxy hóa của dầu cá được đánh giá thông qua chỉ số peroxit. Kết quả cho thấy phương pháp NMKL-No.131 cho hiệu suất thu hồi chất béo cao nhất, nhưng dầu cá thu được bị oxy hóa nhiều. Trong khi đó, phương pháp đun nóng kết hợp ép cơ học tuy cho tỉ lệ thu hồi thấp hơn, nhưng chất lượng dầu cá tốt hơn nhiều, đảm bảo tiêu chuẩn về chỉ số peroxit để sử dụng trong thương mại. Việc bổ sung các chiết xuất chống oxy hóa tự nhiên từ gừng và chè xanh vào hỗn hợp cá trước khi chiết có tác dụng làm giảm chỉ số peroxit của dầu cá thu được rõ rệt. Sự có mặt của các chiết xuất gừng và chè trong dầu cá đã có tác dụng làm chậm quá trình oxy hóa, tác dụng này tương đương tác dụng của chất chống oxy hóa tổng hợp được dùng phổ biến là BHT.

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