Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Farssi & al / Mor. J. Chem. 6 N°4 (2018) 615-622

Effect of temperature on the quality of refined fish co-product oil during bleaching.

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Received 10 Oct 2018,
Revised 05 Nov 2018,
Accepted 10 Nov 2018.

Abstract

The fish co-product oil (sardine and mackerel) is rich in unsaturated fatty acids (65%), however the refining process has an adverse effect on the double bonding of unsaturated fatty acids. This study aimed to develop a better fish co-product oil refining process. The material used in this research was fish co-product oil as a product of the flour and fish oil industry and Tensil 210FF as its adsorbent. The oil was purified in three stages, degumming, neutralization and bleaching. The different bleaching temperatures (50 °C; 90 °C; 120 °C) were applied. The study showed that oil refined at 50 °C to 90 °C gave a better value of acidity (FFA), PV, anisidine number, fatty acid composition and the lowest total oxidation number.

Keywords: *refining, bleaching temperature; fish co-product oil; unsaturated fatty acid.*

1. Introduction

Fish is widely consumed in many parts of the world by humans because it constitutes an important source of proteins, minerals, vitamins and unsaturated essential fatty acids, especially omega-3 [1]. In Morocco Sardine and mackerel are among the most caught species. Their production has increased slightly every year. In Morocco the fish industry is a huge industrial sector that accommodates diverse production processes such as canning fish. this industry generate a large amount of solid wastes and by-products, which often represent more than 50% of the total fish weight [2]. These solid wastes and by-products can be a valuable source for extraction of crude fish oil. Fish oil is known to be an important source of highly unsaturated fatty acids (Polyunsaturated) such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), which play an important role in the prevention of different human diseases. Fortification of foods with such fatty acids is, thus, increasingly recommend [3]. However these two compounds are the two major sensitive elements to the degradation caused by external factors such as oxygen, temperature, and light [4]. Before the used of fish oil in food applications; oxidation products, pigments and anti-flavor compounds must be removed at the refining level. This refining step is done with the aim at improving the quality of this oil, but it had a negative impact on the oxidation stability of fish oil, due to the elimination of natural antioxidants in the crude oil during the refining process [5-6]. A refining process includes four stages; degumming, neutralization, bleaching, and deodorization [7].Degumming is done to separate the gum from the oil fraction. Neutralization aims at reducing levels of free fatty acids, bleaching aims to improve the color quality of the oil and the objective of deodorization is to reduce the unwanted odor from fish oil [8]. The oil processed by high temperatures can damage the polyunsaturated fatty acids bond (EPA and DHA)[7]. Our study relates more particularly of bleaching steps which is the stage that more use heat treatment. The purpose of this study is to find the best method of bleaching temperature to maintain the stability of unsaturated fatty acids in fish oil. The benefit of this research is to look for the optimal conditions of heat treatment at the bleaching stage in order to maintain the stability of the unsaturated fatty acids.

2. Materials and methods

2.1. Material

The raw materials used to extract fish oil in this study were co-products from the canning industry of sardines and mackerel. For refining, the neutralization process is carried out by the addition of NaOH as a function of the initial amount of free fatty acids contained in the oil and bleaching by the addition of Tonsil 500 (earth) as an absorbent with the coal until 'at 5%.For this bleaching step, three heat treatments of the fish oil were compared, 50 °C, 90 °C and 120 °C. The samples of each heat treatment are homogenized and analyzed for the following parameters: acidity, peroxide number, P-anisidine, total oxidation, fatty acid profile and chlorophyll and carotenoid loading.

2.2. Chemicals reagents

All the reagents were of analytical or HPLC grade. 2,2,4-trimethylpentane heptane and isopropanol used in chromatography and cyclohexane used for extinction coefficient determination were purchased from Professional Laboratory (Casablanca, Morocco). Clear and brown-glass bottles were purchased from Cfimusarl (Casablanca, Morocco).

2.3. Analytical determination

2.3.1. Acidity:

Titratable acidity was determined using the International Standard Organization method **[9].** 10 g of oil was dissolved in 80 mL neutralised taste ethanol (96°). Two drops of phenolphthalein (1% in ethanol) were added to the solution.

The solution was then titrated with 0.1N sodium hydroxide (NaOH), previously standardised against hydrochloric acid (HCl). The titrant volume was recorded and the results calculated as a percentage of the oil (expressed as grams of oleic acid per 100 g of oil).

Acidity [%] = (V × N × 282) / 10 × PE

- V: mL consumption at the equivalence point
- N: concentration of the KOH titrant (e.g. 0.1 mol/L)
- PE : sample weight in g
- 282: Molecular weight of oleic acid

2.3.2. Peroxide value:

Peroxide value was determined using the International Standard Organization method, **[10].** 5g of oil was dissolved in acetic acid / 2, 2, 4-trimethylpentane mixture (3:2). To this solution,1 mL of saturated potassium iodide (KI), (70 g KI/40 mL water), was added and shaken for 1 minute. Water (70 mL) was added, followed by approx 0.5 mL of 1 % starch solution (1 g starch/100mL water). The solution was titrated with previously standardised 0.01N sodium thiosulphate ($Na_2S_2O_3$). The volume of titrant was recorded and the peroxide value calculated and reported as MEq of active oxygen/kg oil.

IP [Meq O2 / Kg] = (V × 1000 × N) / PE

- V: mL consumption at the equivalence point
- N: concentration of the sodium thiosulphate (Na2S2O3) titrant (e.g. 0.01 mol/L)
- PE : sample weight in g

2.3.3. Determinationof the Para-anisidine value

Peroxide value was determined using the International Standard Organization method, **[11].**

0.5 to 4.0 g ± 0.001 g of oil are first weighed into a 25 ml flask, and the sample is then diluted with 25 ml of iso-octane and the absorbance (Ab) of the solution is measured at 350 nm, then 5 ml of the fat solution is withdrawn in a first tube and 5 ml of solvent are added to a second tube and 1 ml of the Para-anisidine solution is added to the two tubes. Absorbance (As) of the solvent in the tube at 350 nm.

2.3.4. Carotenoid and Chlorophyll.

The carotenoid and chlorophyll compounds were determined at 470 and 670 nm, respectively, in cyclohexane, using the specific extinction coefficients, according to the method of Minguez-Mosquera et al **[12]**. The values of the specific extinction coefficients used were E0 = 613 for pheophytine as the main component in the chlorophyll fraction and E0 = 2000 for lute in as a major component in the carotenoid fraction. Thus, the pigment content was calculated as follows. Where A is the absorbance and d is the spectrophotometer cell thickness (1 cm). Chlorophyll and carotenoid contents were expressed as mg of pheophytin‘‘a’’ and lute in per kg of oil, respectively:

[Chlorophyll] mg kg-1A670* 106 /613 * 100 * d

[Carotenoid] mg kg-1A470 *106/2000*100 * d

2.3.5. Fatty Acid Composition

Fatty acid composition was determined using the International Standard Organization (Organization) method **[13].** Fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 60 mg oil and 3 mL of hexane with 0.3 mL of 2 N methanolic potassium hydroxide they were analyzed by gas chromatograph (Varian CP-

3800, Varian Inc.) equipped with a FID. The column used was a CP- Wax 52CB column (30 m×0.25 mm i.d.; Varian Inc., Middelburg, The Netherlands).The carrier gas was helium, and the total gas flow rate was 1 ml/min. The initial column temperature was 170 °C, the final temperature 230 °C, and the temperature was increased by steps of 4 °C/min. The injector and detector temperature was 230 °C. Data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA).The results were expressed as the relative percentage of each individual fatty acid (FA) presents in the sample**.**

2.3.6. Statistical Analysis.

Values reported in tables and figures are the means ± SE of three replications.

3. Results and Discussions

3.1. The effect of heat treatment on the free fatty acid content

The acidity of oil is evaluated from the amount of free fatty acids, expressed as grams of oleic acid per 100 g of oil. It is usually used as an indicator for the classification of the different commercial types of oils **[14].** After refining fish oil, the acidity values were 0.17%, 0.20% and 0.32% respectively at temperatures of 50 °C, 90 °C and 120 °C (Figure 1). Compared to the crude oil, the acidity of the purified oil at 50 °C decreased by 94.91%, at 90 °C by 94.01%, while for the oil purified at 120 °C, the decrease was been 90.42%.On the basis of the statistical analysis of the results we noted that the acidity of the oil is very sensitive to the temperature of the bleaching stage of the fish oil refining. At a treatment temperature of 50 °C, the fatty acids are more stable and therefore less oxidizable. The double bonds of unsaturated fatty acids are very sensitive to heat; therefore, a low temperature treatment can preserve its bonds **[15].**

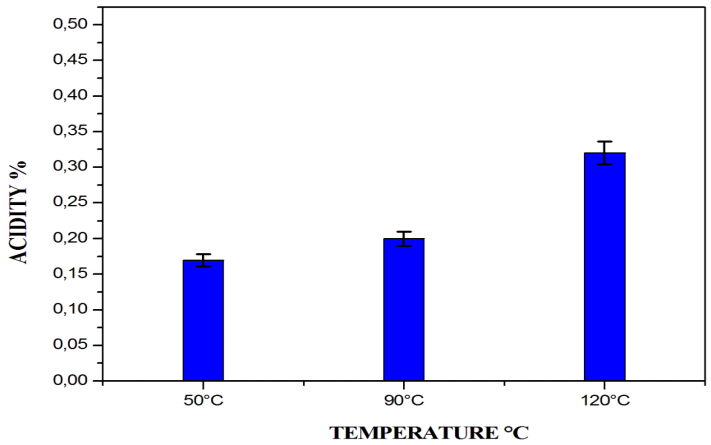

Figure 1: Acidity of refined fish oil in different temperatures.

3.2. The effect of heat treatment on the peroxide value

Oxidation and the formation of peroxides occur during oil extraction and processing and can continue after bottling and during storage. Peroxides are the inter-mediate oxidation products of oil which lead to the formation of a complex mixture of volatile compounds such as aldehydes, ketones, hydrocarbons, alcohols and esters responsible for the deterioration of organoleptic properties **[16].** Therefore, their formation dramatically impacts the shelf life and consumer acceptance of the oil.High temperature and light are two well-known factors that generally promote peroxide formation and degradation **[17].**During the refining at different temperatures, the values of the peroxide value recorded a decrease of 40.5% during the treatment at 50 °C, with a value of 11.48 Meq O_2 / Kg and a decrease of 41.4% at 90 °C with a value of 11.31 Meq O_2 / Kg. On the other hand, the treatment at 120 °C, the peroxide index was 8.98 Meq O_2 / Kg, a decrease of 53.50%. These three values remain non-compliant with the requirement of codex

alimentarus of 5 $MeqO_2$ / Kg for refined fish oil. The lowest value of the peroxide value was recorded at 120 °C. This is explained by the activation of reactions of transformation of primary oxidation compounds into secondary compounds such as aldehydes, ketones, alcohol, during the oil refining at this high temperature of 120 °C.

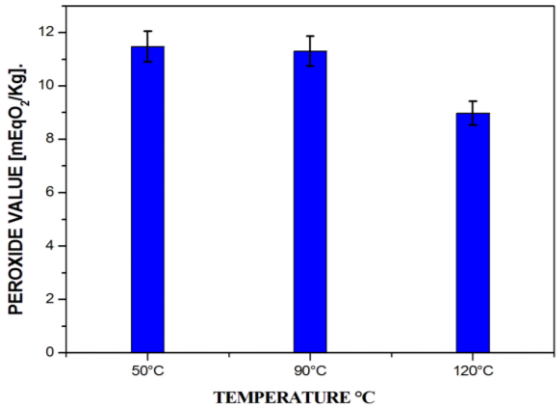

Figure 2: peroxide value of refined fish oil in different temperatures

3.3 The effect of heat treatment on the Para-anisidine value

The oxidation of the oil goes through two stages, a first where we have the formation of hydro peroxides and a second stage where we have a proliferation of hydro peroxides which turn into secondary products of oxidation of aldehydes and ketones **[18].** At this stage, the peroxide index is not sufficient to judge the oxidation state of these samples, and it must be combined with the value of the anisidine index **[19].** The p-anisidine value takes into account the non-volatile aldehyde compounds (long carbon chain aldehydes). It thus makes it possible to evaluate the degree of oxidation of the unsaturated fatty acids of the fat. The p-anisidine values recorded at the various temperatures of the fading step of the present study, it showed a significant variation as a function of the temperature variation. The lowest value was 44.26, recorded at 50 °C, followed by 47.05 at 90 °C and 52.58 at 120 °C.Compared to the crude oil, the p-anisidine value of the refined oil at a temperature of 50 °C decreased by 50.71%. At a temperature of 90 °C, it decreased by 47.60%. At a higher temperature of 120 °C, the decrease was 41.44%.The statistical analysis of these data shows that the lower the temperature, the lower the p-anisidine value, thus giving a better quality of fish co-product oil.

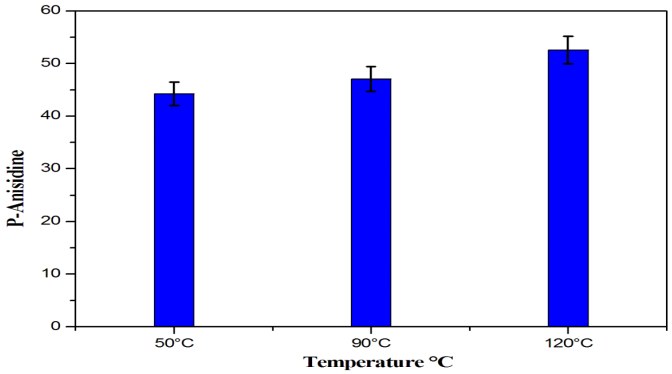

Figure 3: P-anisidine value of refined fish oil in different temperatures

3.4. The effect of heat treatment on the total oxidation value

Total oxidation (TOTOX) value is based on both PV and AV to give a better description of the oxidative status of oils high in PUFA. This represents a measure of the non-volatile carbonyls present in processed oils (PV), together with any further oxidation products which develop during storage (AV) **[20].** The statistical analysis of these data shows that the lower the temperature, the lower the p-anisidine value, thus giving a better quality of fish co-product oil. The

TOTOX value, calculated by the formula P-A + 2PV, makes it possible to determine the overall oxidation state of oil, the higher the temperature, the more this index increases. Thus, at a heat treatment of 90 °C and 120 °C, the values were respectively 69.67 and 70.54. On the other hand, at a temperature of 50 °C, the value was lower of 67.22. Relative to the crude oil, the total oxidation indices of the purified oil decreased by 47.65% at a temperature of 50 °C, 45.75% at a temperature of 90 °C and 45.07% at a temperature of 120 °C.

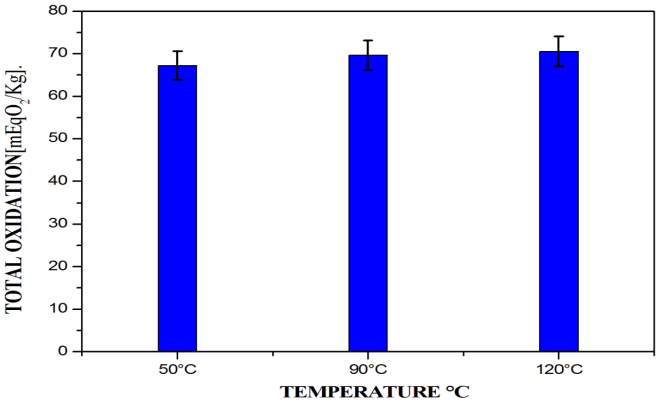

Figure 4: Total oxidation of refined fish oil in different temperature.

3.5 .The effect of heat treatment on the Fatty acid composition

Table1: Fatty acid (%) compositions of refined fish oil in different temperature.

	Crude oil	Different bleaching temperatures		
Fatty Acid		50°C	90°C.	120°C
Lauric Acid C12 : 0	0.1±0.1	0.12±0.1	0.13±0.1	0.17±0.1
Myristic C14 : 0	8.2±0.1	7.8±0.1	7.7±0.1	7.6±0.1
Pentadecanoic Acid C15 : 0	0.1±0.1	0.2±0.1	0.2±0.1	0.1±0.1
PalmiticAcidC16: 0	19.0±0.1	18.4±0.1	18.4±0.1	18.1±0.1
HeptdecanoicAcidC17 : 0	1.8±0.1	1.7±0.1	1.7±0.1	1.7±0.1
Stearic Acid C18 : 0	4.0±0.1	3.7±0.1	3.7±0.1	3.6±0.1
Arachidic Acid C20:0	0.4±0.1	0.1±0.1	0.3±0.1	0.3±0.1
Saturated Fatty Acids (SFA)	33.6±0.1	32.1±0.1	32.2±0.1	31.6±0.1
Pentadecenoic Acid C15 : 1	0.4±0.1	0.5±0.1	0.26±0.1	0.5±0.1
PalmetoleicAcidC16 : 1	8.6±0.1	10.2±0.1	10.1±0.1	10.0±0.1
Heptadecenoic Acid C17 : 1	2.4±0.1	2.6±0.1	2.6±0.1	2.5±0.1
Oleic Acid C18:1	10.4±0.1	11.9±0.1	11.9±0.1	11.9±0.1
Gondoic Acid C20:1	0.3±0.1	0.4±0.1	0.4±0.1	0.6±0.1
Erucic Acid C22:1	3.8±0.1	1.0±0.1	0.6±0.1	1.0±0.1
Monounsaturated Fatty Acid (MUFA)	25.9±0.1	26.8±0.1	25.8±0.1	26.6±0.1
Linoleic Acid C18:2	1.3±0.1	1.2±0.1	1.3±0.1	1.2±0.1
Linolenic Acid C18:3	3.0±0.1	3.1±0.1	3.1±0.1	3.1±0.1
Ecosodienoic Acid C20:2	1.8±0.1	1.3±0.1	1.4±0.1	1.4±0.1
Arachidonic AcidC20:4	1.1±0.1	1.0±0.1	1.0±0.1	1.2±0.1
Ecosapentaenoic Acid C20:5 EPA	21.2±0.1	22.6±0.1	22.5±0.1	22.2±0.1
Docosadienoic Acid C22:2	1.6±0.1	1.8±0.1	1.0±0.1	2.1±0.1
Docosahexanoic Acid C22:6 DHA	10.5±0.1	10.1±0.1	11.5±0.1	10.6±0.1
Polyunsaturated Fatty Acid (PUFA)	40.5±0.1	41.2±0.1	41.9±0.1	43.9±0.1

Fatty acids (FA) are major cellular constituents that form integral parts of the cell membrane and impact the membrane's fluidity and function **[21].** Thus fatty acid composition is an essential indicator of the nutritional value of oil **[22].**Fish oils are often used in animal feed because they are a very good source of energy and n-3 unsaturated fatty acids. Similarly, in human nutrition, these oils are considered the main sources of n-3 fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with well-known positive effects on cardiovascular risk reduction, hypertension, cancer and several inflammatory and autoimmune disorders **[23].** The fatty acid composition of fish oil in different temperature treatment is given in table-1. These results show that this oil is richer in unsaturated fatty acids which total 66.48%,which is twice the level of saturated fatty acids.The three heat treatments used in fish oil bleaching step have led a slight difference in SFA, MUFA and PUFA. These results therefore show that the FA profile is not affected by the refining of fish oil at temperatures of 50 °C, 90 °C and 120 °C. As the method used for this determination is only qualitative, the actual changes in the amount of each fatty acid are not observed. However these results indicate that the changes which are occurring has done at a consistent rate that is, treatment temperature does lead to the breakdown of the individual fatty acid, but at a consistent rate therefore the proportion of fatty acids does not alter, similar to findings by other researchers **[24].**

3.6. The effect of heat treatment on the Carotenoid and chlorophylls.

Changes in the concentration of chlorophylls and carotenes in the fish co-product oil samplesof the three heat treatments 50 °C, 90 °C and 120 °Care shown in table-2. The chlorophyll and carotenoid content of the fish oil was determined by the absorbance measured at wavelengths 670 nm and 470 nm respectively **[12].** The results in Table 2 show that the sample of the crude oil has a high initial charge of chlorophyll pigment, with a concentration of 0.042 ppm. After the bleaching step carried out at different temperatures, this chlorophyll content was significantly decreased at 50 °C, removed 36% of pigments. Treatment at 90 °C resulted in the removal of 62% of pigments, almost double the effect of treatment at 50 °C. The higher heat treatment of 120 °C did not further reduce the chlorophyll content compared to the 90 °C treatment. Carotenoids are highly conjugated terpene compounds of the empirical formula $C_{40}H_{56}$. They absorb strongly in the field of the visible **[25].** They are found under several types (α, β and γ). The most abundant is β-carotene, biochemical precursor of vitamin A. This compound is well known as a deactivator of oxygen and because of this, it is considered one of the most effective inhibitors of photo-oxidation induced by chlorophyll pigments [26-28]. The results show that the crude oil has an initial charge of 1.14 ppm. After refining, there was a significant decrease in all heat treatments in our experiment, a grade of 0.38 ppm at 50 °C, 0.37 ppm at 90 °C, and 0.37 ppm at 120°C. The carotenoid content decreased by 66.66% at 50 °C, 67.0% at 90 °C and 71.92% at a temperature of 120 °C. The removal of these pigments can increase the longevity and stability of oil during storage. For our study, the results showed that the optimal temperature of the discoloration for the removal of these pigments is between 90 and 120 °C.

Table- 2: The chlorophyll and carotenoid absorbance in ppm of fish co-product oil at different temperatures.

Oil			Different bleaching temperatures					
	Crude oil		50°C		90°C		90°C	
λ [nm]	470	670	470	670	470	670	470	670
Content [ppm]	1.14±0.1	0.04±0.01	0.38±0.2	0,03±0.01	0.37±0.1	0,02±0.01	0.37±0.2	0,02±0.01

4. Conclusion

Bleaching the fish by-product oil during refining, at temperatures between 50 °C and 90 °C, allows obtaining better oil quality in terms of acidity, peroxide value, Para-anisidine and the fatty acid profile. The presence of chlorophylls and carotenoid in refined oil play an antioxidant role, On condition that they are stored away from light. We noted a better content of these two pigments (chlorophyll and carotenoid) at 90 ° C in the oil bleaching step. The ideal temperature to be used for the refining fish by-product oil is between 50 °C and 90 °C, because of the pigment value and its stability towards oxidation.

Acknowledgements-This work has been supported in part by the Morocco-French (CNRST-CNRS)

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