IRJSE International Research Journal of Science & Engineering

Int. Res. J. of Science & Engineering, Volume 11 (2) 2023
ISSN: 2322-0015

SJIF Impact Factor 6.70

ORIGINAL ARTICLE OPEN ACCESS

Estimation of Free Fatty Acids, Iodine Value and Saponification Value of Omega-3-Salmon Oil and its FTIR study

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Manuscript Details
Received :13.01.2023
Accepted: 16.03.2023
Published: 07.04.2023

Available online on https://www.irjse.in
ISSN: 2322-0015

Cite this article as:
Gupta Swaroopa Rani N. Estimation of Free Fatty Acids, Iodine Value and Saponification Value of Omega-3-Salmon Oil and its FTIR study, *Int. Res. Journal of Science & Engineering,* 2023, Volume 11(2): 51-62.

Abstract

Omega−3 fatty acids, also called Omega-3 oils, ω−3 fatty acids or *n*−3 fatty acids, are polyunsaturated fatty acids (PUFAs) characterized by the presence of a double bond, three atoms away from the terminal methyl group in their chemical structure. They are widely distributed in nature, being important constituents of animal lipid metabolism, and they play an important role in the human diet and in human physiology. The three types of omega−3 fatty acids involved in human physiology are α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALA can be found in plants, while DHA and EPA are found in algae and fish. Marine algae and phytoplankton are primary sources of omega−3 fatty acids. DHA and EPA accumulate in fish that eat these algae. Common sources of plant oils containing ALA include walnuts, edible seeds, and flaxseeds, while sources of EPA and DHA include fish and fish oils, as well as algae oil. Mammals are unable to synthesize the essential omega−3 fatty acid ALA and can only obtain it through diet. However, they can use ALA, when available, to form EPA and DHA, by creating additional double bonds along its carbon chain (desaturation) and extending it (elongation). Namely, ALA (18 carbons and 3 double bonds) is used to make EPA (20 carbons and 5 double bonds), which is then used to make DHA (22 carbons and 6 double bonds). The ability to make the longer-chain omega−3 fatty acids from ALA may be impaired in aging. In foods exposed to air, unsaturated fatty acids are vulnerable to oxidation and rancidity. Present Paper deals with Estimation of Free Fatty acids of Omega 3 Salmon Oil, Estimation of Iodine value of Omega 3 Salmon Oil, Estimation of Saponification value of Omega 3 Salmon Oil and FTIR study of Omega 3 Salmon Oil.

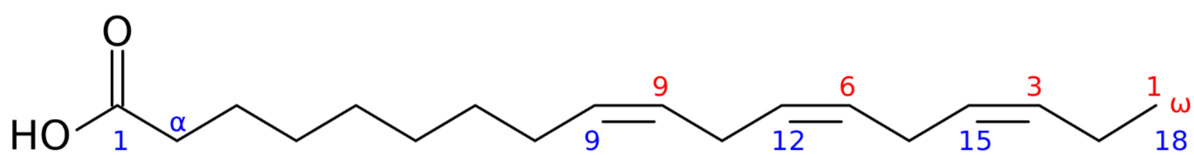
Key words: Omega-3-Salmon Oil, Estimation of Free Fatty acids, Estimation of Iodine value, Estimation of Saponification value, FTIR study

1. Introduction

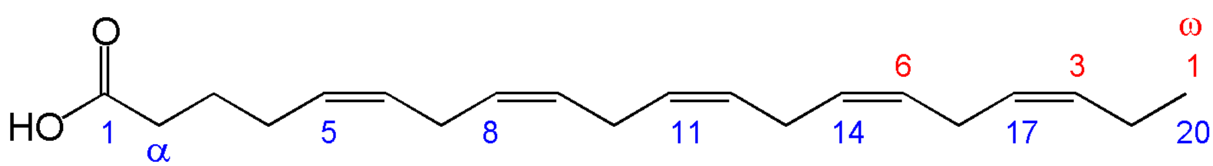
Omega-3 oils

Omega−3 fatty acids, also called Omega-3 oils, ω−3 fatty acids or *n*−3 fatty acids, are polyunsaturated fatty acids (PUFAs) characterized by the presence of a double bond, three atoms away from the terminal methyl group in their chemical structure. They are widely distributed in nature, being important constituents of animal lipid metabolism, and they play an important role in the human diet and in human physiology. The three types of omega−3 fatty acids involved in human physiology are α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALA can be found in plants, while DHA and EPA are found in algae and fish. Marine algae and phytoplankton are primary sources of omega−3 fatty acids. DHA and EPA accumulate in fish that eat these algae. Common sources of plant oils containing ALA include walnuts, edible seeds, and flaxseeds, while sources of EPA and DHA include fish and fish oils, as well as algae oil. Mammals are unable to synthesize the essential omega−3 fatty acid ALA and can only obtain it through diet. However, they can use ALA, when available, to form EPA and DHA, by creating additional double bonds along its carbon chain (desaturation) and extending it (elongation). Namely, ALA (18 carbons and 3 double bonds) is used to make EPA (20 carbons and 5 double bonds), which is then used to make DHA (22 carbons and 6 double bonds). The ability to make the longer-chain omega−3 fatty acids from ALA may be impaired in aging. In foods exposed to air, unsaturated fatty acids are vulnerable to oxidation and rancidity. [1-8]

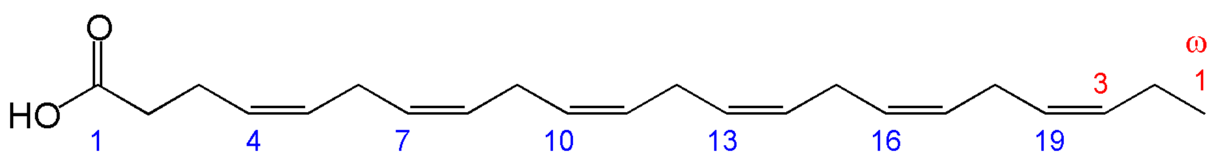
There is no high-quality evidence that dietary supplementation with omega−3 fatty acids reduces the risk of cancer or cardiovascular disease. Furthermore, fish oil supplement studies have failed to support claims of preventing heart attacks or strokes or any vascular disease outcomes. [9-14].



Chemical structure of α-linolenic acid (ALA), a fatty acid with a chain of 18 carbons with three double bonds on carbons numbered 9, 12, and 15. The omega (ω) end of the chain is at carbon 18, and the double bond closest to the omega carbon begins at carbon 15 = 18−3. Hence, ALA is a ω−3 fatty acid with ω = 18. [15]



Chemical structure of eicosapentaenoic acid (EPA) [16]



Chemical structure of docosahexaenoic acid (DHA) [17]

Rancidity

A 2022 study found that a number of products on the market used oxidised oils, with the rancidity often masked by flavourings. Another study found that 2015 an average of 20% of products had excess oxidation. Whether rancid fish oil is harmful remains unclear. Some studies show that highly oxidised fish oil can have negative impact on cholesterol levels. Animal testing showed that high doses have toxic effects. Furthermore, rancid oil is likely to be less effective than fresh fish oil. [18, 19].

Fish

The most widely available dietary source of EPA and DHA is oily fish, such as salmon, herring, mackerel, anchovies, and sardines. Oils from these fishes have around seven times as much omega−3 as omega−6. Other oily fish, such as tuna, also contain *n*-3 in somewhat lesser amounts. Although fish are a dietary source of omega−3 fatty acids, fish do not synthesize omega−3 fatty acids, but rather obtain them via their food supply, including algae or plankton. In order for farmed marine fish to have amounts of EPA and DHA comparable to those of wild-caught fish, their feed must be supplemented with EPA and DHA, most commonly in the form of fish oil. For this reason, 81% of the global fish oil supply in 2009 was consumed by aquaculture. [20-22].

Fish oil

Marine and freshwater fish oil vary in content of arachidonic acid, EPA and DHA. They also differ in their effects on organ lipids. Not all forms of fish oil may be equally digestible. Of four studies that compare bioavailability of the glyceryl ester form of fish oil vs. the ethyl ester form, two have concluded the natural glyceryl ester form is better, and the other two studies did not find a significant difference. No studies have shown the ethyl ester form to be superior, although it is cheaper to manufacture. [23-25]

Krill

Krill oil is a source of omega−3 fatty acids. The effect of krill oil, at a lower dose of EPA + DHA (62.8%), was demonstrated to be similar to that of fish oil on blood lipid levels and markers of inflammation in healthy humans. While not an endangered species, krill are a mainstay of the diets of many ocean-based species including whales, causing environmental and scientific concerns about their sustainability. Preliminary studies appear to indicate that the DHA and EPA omega−3 fatty acids found in krill oil may be more bio-available than in fish oil. Additionally, krill oil contains astaxanthin, a marine-source keto-carotenoid antioxidant that may act synergistically with EPA and DHA. [26-35].

Plant sources

Table 1. ALA content as the percentage of the seed oil. [36]

Common name	Alternative name	Linnaean name	% ALA
kiwifruit (fruit)	Chinese gooseberry	*Actinidia deliciosa*	63
perilla	shiso	*Perilla frutescens*	61
chia	chia sage	*Salvia hispanica*	58
linseed	flax	*Linum usitatissimum*	53 – 59
lingonberry	cowberry	*Vaccinium vitis-idaea*	49
fig	common fig	*Ficus carica*	47.7
camelina	gold-of-pleasure	*Camelina sativa*	36
purslane	portulaca	*Portulaca oleracea*	35
black raspberry		*Rubus occidentalis*	33
hempseed		*Cannabis sativa*	19
canola	rapeseed	mostly *Brassica napus*	9 – 11

Table 2. ALA content as the percentage of the whole food. [37, 38]

Common name	Linnaean name	% ALA
linseed	*Linum usitatissimum*	18.1
hempseed	*Cannabis sativa*	8.7
butternut	*Juglans cinerea*	8.7
Persian walnut	*Juglans regia*	6.3
pecan	*Carya illinoinensis*	0.6
hazelnut	*Corylus avellana*	0.1

Linseed (or flaxseed) (*Linum usitatissimum*) and its oil are perhaps the most widely available botanical source of the omega−3 fatty acid ALA. Flaxseed oil consists of approximately 55% ALA, which makes it six times richer than most fish oils in omega−3 fatty acids. A portion of this is converted by the body to EPA and DHA, though the actual converted percentage may differ between men and women. In 2013 Rothamsted Research in the UK reported they had developed a genetically modified form of the plant Camelina that produced EPA and DHA. Oil from the seeds of this plant contained on average 11% EPA and 8% DHA in one development and 24% EPA in another. [39-42]

Eggs

Eggs produced by hens fed a diet of greens and insects contain higher levels of omega−3 fatty acids than those produced by chickens fed corn or soybeans. In addition to feeding chickens insects and greens, fish oils may be added to their diets to increase the omega−3 fatty acid concentrations in eggs. The addition of flax and canola seeds to the diets of laying, both good sources of alpha-linolenic acid, increases the omega−3 content of the eggs, predominantly DHA. However, this enrichment could lead to an increment of lipid oxidation in the eggs if the seeds are used in higher doses, without using an appropriate antioxidant. The addition of green algae or seaweed to the diets boosts the content of DHA and EPA, which are the forms of omega−3 approved by the FDA for medical claims. A common consumer complaint is "Omega−3 eggs can sometimes have a fishy taste if the hens are fed marine oils". [43-47]

Meat

Omega−3 fatty acids are formed in the chloroplasts of green leaves and algae. While seaweeds and algae are the sources of omega−3 fatty acids present in fish, grass is the source of omega−3 fatty acids present in grass-fed animals. When cattle are taken off omega−3 fatty acid-rich grass and shipped to a feedlot to be fattened on omega−3 fatty acid deficient grain, they begin losing their store of this beneficial fat. Each day that an animal spends in the feedlot, the amount of omega−3 fatty acids in its meat is diminished. The omega−6:omega−3 ratio of grass-fed beef is about 2:1, making it a more useful source of omega−3 than grain-fed beef, which usually has a ratio of 4:1. In a 2009 joint study by the USDA and researchers at Clemson University in South Carolina, grass-fed beef was compared with grain-finished beef. The researchers found that grass-finished beef is higher in moisture content, 42.5% lower total lipid content, 54% lower in total fatty acids, 54% higher in beta-carotene, 288% higher in vitamin E (alpha-tocopherol), higher in the B-vitamins thiamin and riboflavin, higher in the minerals calcium, magnesium, and potassium, 193% higher in total omega−3s, 117% higher in CLA (cis-9, trans-11 octadecenoic acid, a conjugated linoleic acid, which is a potential cancer fighter), 90% higher in vaccenic acid (which can be transformed into CLA), lower in the saturated fats, and has a healthier ratio of omega−6 to omega−3 fatty acids (1.65 vs 4.84). Protein and cholesterol content were equal. The omega−3 content of chicken meat may be enhanced by increasing the animals' dietary intake of grains high in omega−3, such as flax, chia, and canola.

Kangaroo meat is also a source of omega−3, with fillet and steak containing 74 mg per 100 g of raw meat. [48-52]

Seal oil

Seal oil is a source of EPA, DPA, and DHA, and is commonly used in Arctic regions. According to Health Canada, it helps to support the development of the brain, eyes, and nerves in children up to 12 years of age. Like all seal products, it is not allowed to be imported into the European Union. [53, 54]

Other sources

A trend in the early 21st century was to fortify food with omega−3 fatty acids. The microalgae *Crypthecodinium cohnii* and *Schizochytrium* are rich sources of DHA, but not EPA, and can be produced commercially in bioreactors for use as food additives. Oil from brown algae (kelp) is a source of EPA. The alga *Nannochloropsis* also has high levels of EPA. [55-58]

Present Paper deals with Estimation of Free Fatty acids of Omega 3 Salmon Oil, Estimation of Iodine value of Omega 3 Salmon Oil, Estimation of Saponification value of Omega 3 Salmon Oil and FTIR study of Omega 3 Salmon Oil.

2. Methodology

1. Estimation of Free Fatty acids of Omega 3 Salmon Oil

Fat or oil contain small quantity of free fatty acids. On storing, the free fatty acid contents of the fat or oil increases. The free fatty acid contents are determined by direct titration of fat or oil with standard KOH solution. The acid value is defined as the numbers of milligrams of KOH required to neutralize the free fatty acid present in 1 g of the fat or oil.

$RCOOH + KOH \rightarrow RCOOK + H2O$

Preparation of 0.1 N Oxalic Acid: 0.63 g of oxalic acid was dissolved in 100ml of distilled water.

Preparation of 0.1 N Potassium Hydroxide: 0.56 g of KOH was dissolved in 100 ml of distilled water.

Standardization of KOH: 10 ml of oxalic acid was taken in 100ml of volumetric flask and 2-3 drops of phenolphthalein indicator was added. Then titrated with 0.1 N KOH. At end point it turns colourless to pink.

Titration of Omega-3 Salmon oil: 1gm of Omega-3 Salmon oil was taken in 100 ml of conical flask, 5ml alcohol and 2-3 drops of phenolphthalein indicator was added. Then titrated with 0.1 N KOH. At end point it turns colourless to pink.

2. Estimation of Iodine value of Omega 3 Salmon Oil

The iodine value of oil is the number of grams of iodine taken up by 100 g of the oil. It is determined by reacting a known volume of excess solution of iodine monochloride in acetic acid (Wij's solution) with oil and then back titrating unreacted iodine with Sodium thiosulphate solution.

Preparation of Sodium thiosulphate: 2.48 g of sodium thiosulphate was dissolved in 100 ml of distilled water.

Preparation of Standard $CuSO_4$: In 100 ml volumetric flask 1.995 g of $CuSO_4$ was taken and dissolved in distilled water and to it 3-4 drops of concentrated H_2SO_4 was added and volume was made upto 100 ml with distilled water.

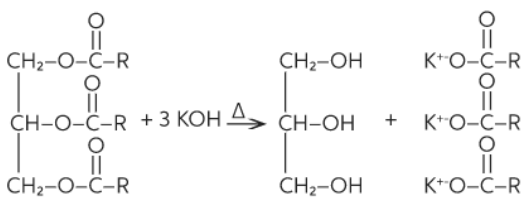
Standardization of Sodium thiosulphate: 10 ml Standard $CuSO_4$ was taken in 100 ml of conical flask and then NH_4OH was added till turbidity appeared. Then dilute acetic acid was added till the turbidity vanished. And 10 ml 10% of KI was added. Then it was titrated with sodium thiosulphate till pale yellow colour appeared. Then 1 ml of starch solution was added and titration was continued with sodium thiosulphate, till blue colour converted into white, then pinch of ammonium thiocyanate was added, blue colour was appeared then again titrated with Sodium thiosulphate till the blue colour turned into white.

Titration of Omega 3 Salmon Oil: 1.42 gm oil, 20ml of Carbon tetrachloride and 30ml Wij's solution was taken in 250ml of conical flask and stoppered the flask. It was then shaken and allowed it to stand in the dark for 1 hour with occasional shaking. 10ml of 10% KI, 60ml of distilled and 2-3 ml of freshly prepared starch solution was added and then titrated with Sodium thiosulphate till the colour disappeared. Noted the end point.

Blank Titration: Blank titration was performed without taking oil. Noted the end point.

3. Estimation of Saponification value of Omega 3 Salmon Oil

Saponification value of an oil or fat is defined as the number of milligrams of Potassium Hydroxide required to hydrolyse (saponify) one gram of an oil completely. A known amount of oil is refluxed with excess amount of standard alcoholic potash solution and the unused alkali is titrated against standard acid solution using phenolphthalein as an indicator. The reaction is as follows:



Preparation of Standard Oxalic acid: 1.65 g of the Oxalic acid was dissolved in 100 Distilled water.

Saponification of Omega 3 Salmon Oil: 0.707 g of given Omega 3 Salmon Oil was taken in a 250 ml conical flask. To it 10 ml of 0.5 N KOH solution was added. Air condenser was fitted to the conical flask. The reaction mixture was refluxed on a boiling water bath for half an hour. The resulting solution was cooled and titrated it against standard oxalic acid solution. Noted the end point.

Blank Titration: Titration was performed using blank solution (without oil) and noted the end point.

4. FTIR study of Omega 3 Salmon Oil

FTIR can be routinely used to identify the functional groups and identification/quality control of raw material/finished products. Spectrum RX-I offers fast throughput and rapid access to reliable and dependable IR results. High signal to noise ratio makes FTIR more useful for difficult samples. It has resolution of 1 cm1 and scan range of 4000 cm-1 to 250 cm-1. In the normal mode around 10 mg sample is required in the form of fine powder. The sample can be analyzed in the form of liquid, solid and thin films also.

FTIR spectra of Omega - 3 Salmon Oil is obtained at room temperature by using an FTIR Spectrophotometer - Perkin Elmer - Spectrum RX- IFTIR. The spectra is collected in range from 432.05 - 3741.90 cm^{-1}.

3. Results and Discussion

1. Estimation of Free Fatty acids of Omega 3 Salmon Oil

Standardization of KOH

S.N.	Volume of oxalic acid taken	Volume of 0.1N KOH required	Constant reading V1
1.	10ml	20 ml	20 ml
2.	10ml	20ml	
3.	10ml	20 ml	

Titration of Omega-3 Salmon oil

S.N.	Volume of alcohol	Volume of 0.1N KOH	Constant reading V2
1.	5 ml	6.5 ml	6.5 ml
2.	5 ml	6.5 ml	
3.	5 ml	6.5 ml	

2. Estimation of Iodine value of Omega 3 Salmon Oil

Standardization of Sodium thiosulphate

Volume of $CuSO_4$	Vol. of Sodium thiosulphate	Constant reading V1
10 ml	9.1 ml	9.1 ml
10 ml	9.1 ml	
10 ml	9.1 ml	

Titration of Omega 3 Salmon Oil

Omega 3 Salmon Oil	Vol. of Sodium thiosulphate V2
1.42 g	12 ml

Blank Titration

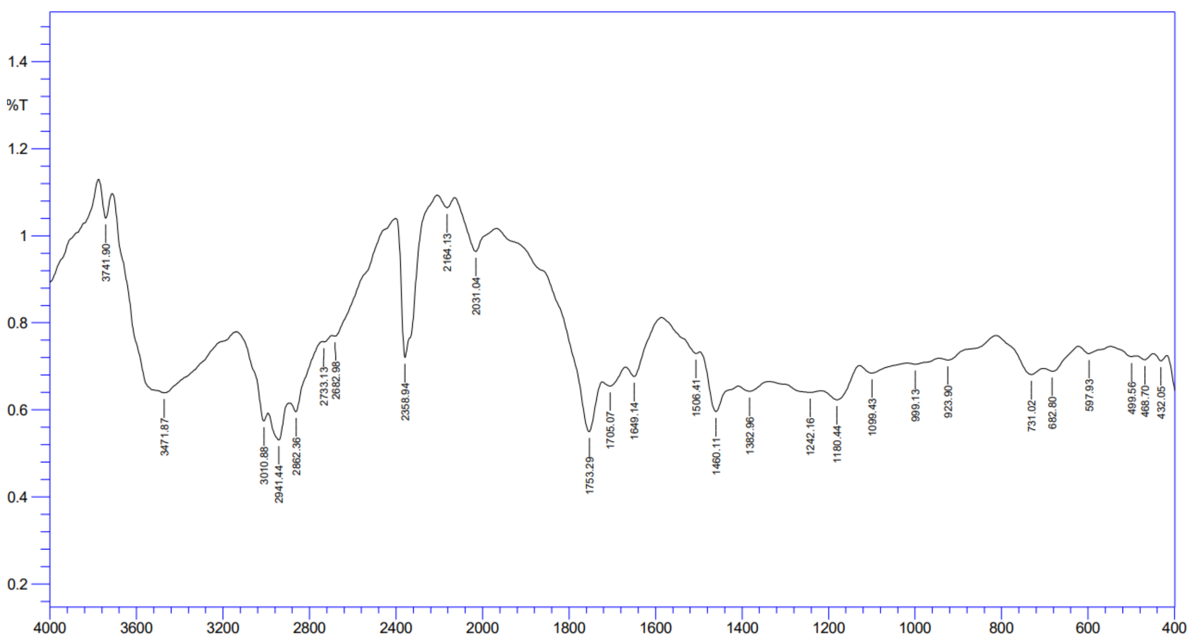
Omega 3 Salmon Oil	Vol. of Sodium thiosulphate V3
0 g	28 ml

3. Estimation of Saponification value of Omega 3 Salmon Oil

Volume of standard Oxalic acid used for blank Titration (V1) = 28 ml

Volume of standard Oxalic acid used for Omega 3 Salmon Oil Titration (V2) = 27 ml

4. FTIR study of Omega 3 Salmon oil



1. Estimation of Free Fatty acids of Omega 3 Salmon Oil

Normality of oxalic acid (N1) = $\frac{Weight\ of\ Oxalic\ Acid \times 10}{63} = \frac{0.63 \times 10}{63} = 0.1$

Normality of potassium hydroxide (N2) = $\frac{N1 \times 10}{V1} = \frac{0.1 \times 10}{20} = 0.05$

1 litre 1N KOH ≡ 56 g KOH

V2 ml N2 N KOH $\equiv \frac{V2 \times 56}{1000} \times \frac{N2}{1}$ g KOH
$\equiv$ V2 × 56 × N2 mg KOH
$\equiv$ 6.5 × 56 × 0.05 mg KOH
$\equiv$ 18.2 mg KOH

18.2 mg KOH is required to Neutralize the free fatty acid present in 1g of oil. Hence the acid value of Omega 3 Salmon Oil is 18.2.

2. Estimation of Iodine value of Omega 3 Salmon Oil

Normality of $CuSO_4$ Solution (N1) $= \frac{1.995 \times 10}{249.5} = 0.08$

Normality of Sodium Thiosulphate Solution (N2) $= \frac{N1 \times 10}{V1} = \frac{0.08 \times 10}{9.1} = 0.0879$

Iodine Value $= \frac{(V3-V2) \times N2 \times 127 \times 100}{Wt\ of\ Sample \times 1000} = \frac{(28-12) \times 0.0879 \times 127 \times 100}{1.42 \times 1000} = 12.5784$

Hence the Iodine value of Omega 3 Salmon Oil was found to be 12.5784.

3. Estimation of Saponification value of Omega 3 Salmon Oil

Normality of Oxalic Acid $= \frac{Wt\ of\ Oxalic\ acid\ X10}{63} = \frac{1.65\ X\ 10}{63} = 0.2619$

Volume of Oxalic Acid used = (V1-V2) = (28-27) = 1 ml

1 liter of 1 N KOH $\equiv$ 56g KOH

Hence (V1-V2) ml of 0.5N KOH $\equiv \frac{56\ (V1-V2) X\ 0.5}{1000\ X\ 1}$

$\equiv \frac{56 \times (28-27)\ X\ 0.5}{1000 \times 1} = 0.028$ g KOH = 28 mg KOH

28mg KOH is required to saponify 0.707 g Omega 3 Salmon oil

Hence 1 X 28 / 0.707 i.e. 39.604 mg KOH will be required to saponify 1 g Omega 3 Salmon oil. Therefore, Saponification value of Omega 3 Salmon Oil was found to be 39.604.

4. FTIR study of Omega 3 Salmon oil

Interpretation of IR spectra of Omega 3 Salmon oil is done as follows

S.N.	Spectra region wave number cm^{-1}	Bond causing Absorption	Compound Class	Pattern and intensity of bond
1	3741.90	-	-	Low, Sharp intensity
2	3471.87	O-H Stretching	Alcohol	Strong And Broad intensity
3	3010.88	C-H Stretching	Alkene	Strong And Sharp intensity
4	2941.44	O-H Stretching N-H Stretching	Carboxylic Acid Amine Salt	Strong And Broad intensity
5	2862.36	O-H Stretching N-H Stretching	Carboxylic Acid Amine Salt	Strong And Broad intensity
6	2733.13	C-H Stretching	Aldehyde	Moderate And Broad intensity
7	2682.98	O-H Stretching	Carboxylic Acid	Moderate And Broad intensity
8	2358.94	O-H Stretching	Alcohol	Moderate And Broad intensity
9	2164.13	C≡C Stretching	Alkyne	Low And Broad intensity
10	2031.04	-	-	Low And Broad intensity

11	1753.29	C=O Stretching	Esters	Strong And Broad intensity
12	1705.07	C-H Bending	Aromatic Compound	Low And Broad intensity
13	1649.14	C-H Bending	Aromatic Compound	Low And Broad intensity
14	1506.41	-	-	Low And Broad intensity
15	1460.11	C-H Bending	Alkane	Moderate And Sharp intensity
16	1382.96	-	-	Low And Broad intensity
17	1242.16	-	-	Low And Broad intensity
18	1180.44	-	-	Low And Broad intensity
19	1099.43	-	-	Low And Broad intensity
20	999.13	-	-	Low And Broad intensity
21	923.90	-	-	Low And Broad intensity
22	731.02	-	-	Low And Broad intensity
23	682.80	-	-	Low And Broad intensity
24	597.93	-	-	Low And Broad intensity
25	499.56	-	-	Low And Broad intensity
26	468.70	-	-	Low And Broad intensity
27	432.05	-	-	Low And Broad intensity

Interpretation of FTIR Spectra of Omega-3 Salmon Oil shows presence of various functional groups such as O-H Stretching – Alcohol, O-H Stretching - Carboxylic Acid, C-H Stretching - Alkene, C-H Stretching – Aldehyde, C-H Bending – Alkane, C-H Bending - Aromatic Compound, N-H Stretching - Amine Salt, C≡C Stretching – Alkyne, C=O Stretching – Esters.

4. Conclusion

The acid value of Omega 3 Salmon Oil is 18.2, The Iodine value of Omega 3 Salmon Oil was found to be 12.6 and Saponification value of Omega 3 Salmon Oil was found to be 39.6.

Interpretation of FTIR Spectra of Omega-3 Salmon Oil shows presence of various functional groups such as O-H Stretching – Alcohol, O-H Stretching - Carboxylic Acid, C-H Stretching - Alkene, C-H Stretching – Aldehyde, C-H Bending – Alkane, C-H Bending - Aromatic Compound, N-H Stretching - Amine Salt, C≡C Stretching – Alkyne, C=O Stretching – Esters.

Conflicts of interest: The author stated that no conflicts of interest.

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