

**EFFECT OF TEMPERATURE, TIME AND VOLUME OF SOLVENT USED IN EXTRACTION
SALMON LIVER OIL**

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ABSTRACT

Salomon liver oil is an accessible and easy source of long-chain polyunsaturated fatty acids (PUFAs). These fatty acids play critical role in human health and have significant impacts on prevention from cardiovascular diseases, hypertension, etc. salmon liver oil, as one of the valuable fish residuals, is used in this work for extraction of oil. Current study firstly was done using Soxhlet system due to solvent method, and normal hexane solution was utilized for oil extraction, and the effect of temperature, time and volume of solvent used parameters to extract the amount of oil extracted from salmon liver was examined. Oil extraction process was conducted in temperatures 50, 60, and 68 °C in times 1, 2, 3, and 4 hours. Changes in volume of solvent (150, 200, and 250 ml) were investigated in optimal time and temperature conditions. Finally, direct impact of time and temperature increase, and in a lower degree, direct impact of solvent volume increase on increased amount of oil extracted from salmon liver was specified. Optimal parameters for achieving highest amount of salmon liver oil extraction were temperature 68 °C and 4 hours with solvent volume of 150 ml. Maximum oil extracted under this condition was 2.9711 g from 5 g of original sample (powder of salmon liver).

KEYWORDS: Oil Extraction, Optimal Extraction Conditions, Salmon Liver, Soxhlet System.

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) have attracted attention of many authors over two recent decades due to the pharmacological and nutritive properties available in them (Razak *et al.*, 2001). Consumption of fish as a nutritive material is highly common in most regions of the world because of geographical conditions, where have access to this nutritive source (Schacky and Weber, 1986). According to studies, people who use adequate amount of fish suffer from cardiovascular diseases and related diseases less than those who do not use fish. Omega-3 fatty acids in fish oil are the main factor in this regards (Nardini *et al.*, 1995). Fish liver oil is significant in terms of nutritive and pharmacological value and in addition to inclusion of essential unsaturated fatty acids such as omega-3 fatty acids and arachidonic acid (AA) it also contains high levels of fat-soluble vitamins, A, and D (Anon, 1988). Basic roles of vitamin A in body include production of visual pigment and thus preventing from night blindness (DeSouza *et al.*, 1997).

On the other hand, vitamin D is also necessary for construction of natural bones and facilitates absorption of calcium and phosphorus from the intestine. Putting calcium in the bone tissue is the result of this vitamin action (Schmidt, 1930). Among various type of fish, salmon is considered as fatty fish, thus it is a rich source of omega-3 polyunsaturated essential fatty acids. Also, using the oil extracted from such fish as salmon, which have rapid growth and possibility of heavy metals absorption in them is less dangerous, is important, and it increase necessity for consumption of this type of fish (Lemerond, 2008). On the other hand, salmon is well grown in regional conditions of Iran and it is regarded as the commonly used fish in the community, indicating its easy access and low cost for this valuable nutritive source. Almost one fourth of the total fishing is wasted in the world which is considered as fish wastes. Generally some sections of fish such as skin, liver, head, blades, bone, and offal are disposed in fish processing in the fisheries industry (Falch *et al.*, 2006), while fish liver is an important part of the fish wastes containing high levels of fat-soluble vitamins and polyunsaturated essential fatty acids of omega family (omega-3, omega-6, and omega-9) (Aryee and Simpson, 2009). Fish liver oil possesses many pharmacological and medical properties and can improve some diseases and problems in today communities which are created due to inappropriate nutrition and mental pressures. For example, unsaturated fatty acids in fish oil liver play considerable role in evolution of brain and nerve tissue of infants. This oil is mainly processes and prepared as dry pills (endocapsular) and recommended by the

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physicians, since it reduces risk for cardiovascular diseases, prostate cancer, colon cancer, and breast cancer. It also is useful in reduction of Rheumatoid arthritis (RA) and contributes to the health of the fetus during pregnancy (Shahid Madani *et al.*, 2011). There are various methods for fish oil and fish liver oil extraction. One of common methods for fish oil extraction is Soxhlet system. In order to implement oil extraction, this system requires an organic solvent. In fact, extraction technique using solvent – Soxhlet is one of the most common methods for separation of lipids from nutritive products (including fish) and specifying total content of nutritive material's lipid (McClements, 2003). Non-polar lipids (triacylglycerols, and triglycerides) have higher solubility in non-polar solvents such as hexane, thus Soxhlet system is usually used with normal hexane solvent for oil extraction. Extraction with hexane is one of the common methods for determining total lipids in the dried samples. Therefore, it requires initial pretreatment of samples (drying the samples) for better penetration of the solvent in the sample and better extraction efficiency (Shamsudin and Salimon, 2006). Operational parameters affecting extraction with Soxhlet – normal hexane solvent include temperature, time of extraction operation, and volume of normal hexane parameters. Investigating impact of these parameters on their extraction and optimization helps achieving optimal conditions for achieving maximum oil extracted. Current work aims at investigating and estimating these parameters by some experiments and their influence on among of fish oil extracted from salmon liver.

MATERIALS AND METHODS

Materials and Equipment Used

Materials used in this research include normal hexane solvent owned by Merck of Germany, nitrogen gas or N₂, liquid paraffin and preparing salmon liver. The equipment used include Soxhlet system equipped with a condenser and water inlet and outlet, 100 ml extraction tank, 1000 ml two-span balloon, mercury thermometer, oil bath, scale calibrated by Company Kern, Germany, Electric Heater Manufactured by Heidolph Company, Germany, Rotary Manufactured by Heidolph Company, Germany, Freeze-Dryer machine Manufactured by CHRist Company, Model 1-4LD with a working range of 0.05 mbar under vacuum and cooling rate of 55 °C.

Methodology

Sample Preparation: in order to implement extraction operation, first it is necessary to prepare needed sample (salmon liver). To this end, 1,500 g of salmon liver was prepared and collected among a specific statistical population of fish with average weight and length of 800 g and 31 cm from Tehran fish market in winter. Livers were rinsed and dried in -23 °C, and were stored in freezer for two days. Then, frozen samples were transferred to laboratory complex in Islamic Azad University, Science and Research Branch. They were cut in thick layers and placed in Petri dishes and their surface was covered by aluminum foil. Samples were placed in freeze dryer in the laboratory in two stages. This is for drying fish liver to eliminate moisture in the fish liver, resulting in easier and better for extracting oil from salmon liver. After drying, the lost moisture in 1,500 g of salmon liver was estimated, and the moisture content of 86.66% was reported. Livers of dried fish were used in extraction process by Soxhlet system and normal hexane solvent following turning to dry fish liver powder.

Implementing Experiments:

Soxhlet system using normal hexane solvent was used in order to implement extraction process. In order to implement extraction operation, 5 g of salmon liver powder was weighted each time by laboratory calibrated scale, and was used in extraction tank of Soxhlet system following placement inside thimble. Oil bath prepared by liquid paraffin was used to indirectly transfer heat from electric heater to two-span balloon. Temperature adjustment was done by the electric heater and temperature control to maintain the temperature of the oil extraction operations on the desired temperature was done by a mercury thermometer placed in the side mouth of the two-spa balloon. Amount of the solvent volume used for investigating temperature and time parameters was 150 ml, which was poured on thimble of sample content from extraction tank of Soxhlet system, and syphon action was done inside 100 ml extraction tank, and then condenser was placed on the extraction tank, and oil extraction process was performed at varying temperatures and times. Oil extraction operation for investigating temperature and time parameters was done in temperatures 50, 60, and 68 °C and times 1, 2, 3, and 4 hours, respectively. Following implementing each of the respective experiments and stop of Soxhlet system, the solvent containing extracted oil was separated by rotary machine at temperature range 65 – 68 °C and revolution 90 rpm for 20 – 30 min. Amount of the oil was weighted and percent of fish liver oil extraction was calculated and reported by following equation:

$$\%E_{\text{fish liver oil}} = \frac{W_2 - W_1}{W_3} \times 100 \quad \text{Eq. 1}$$

Where, W_1 is weight of empty dish, W_2 is weight of dish containing oil, and W_3 is initial sample weight (5 g).

RESULTS AND DISCUSSION

Results taken from investigation of temperature and time variables following salmon liver oil extraction operation are given in Tables 1- 4. Considering values reported in the tables, it is clear that amount of oil extracted is increased by increasing temperature from 50 °C to 68 °C in each of considered times, denoting direct impact of temperature increase on increasing percent of oil extraction.

Table 1: Results taken from salmon liver oil extraction during fixed time (1 h)

Percent of fish liver oil extraction	Average fish liver oil extraction (g)	Temperature (°C)	Time (h)
16.386 %	0.8193	50	1
19.718 %	0.9859	60	1
39.230 %	1.9615	68	1

Table 2: Results taken from salmon liver oil extraction during fixed time (2 h)

Percent of fish liver oil extraction	Average fish liver oil extraction (g)	Temperature (°C)	Time (h)
18.596 %	0.9298	50	2
21.084 %	1.0542	60	2
49.830 %	2.4914	68	2

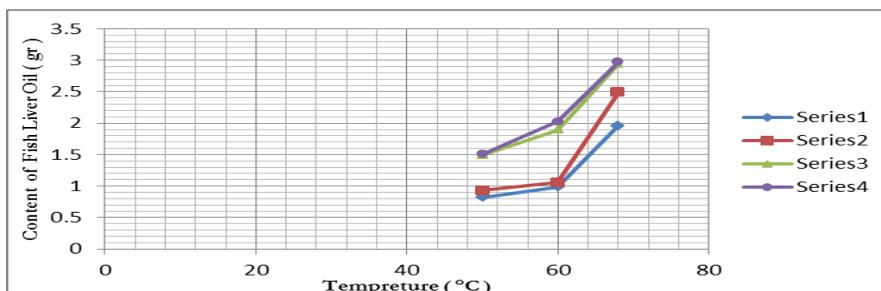
Table 3: Results taken from salmon liver oil extraction during fixed time (3 h)

Percent of fish liver oil extraction	Average fish liver oil extraction (g)	Temperature (°C)	Time (h)
29.732%	1.4866	50	3
37.791%	1.8895	60	3
58.831%	2.9414	68	3

Table 4: Results taken from salmon liver oil extraction during fixed time (4 h)

Percent of fish liver oil extraction	Average fish liver oil extraction (g)	Temperature (°C)	Time (h)
30.145%	1.5072	50	4
40.488	2.0244	60	4
59.423%	2.9711	68	4

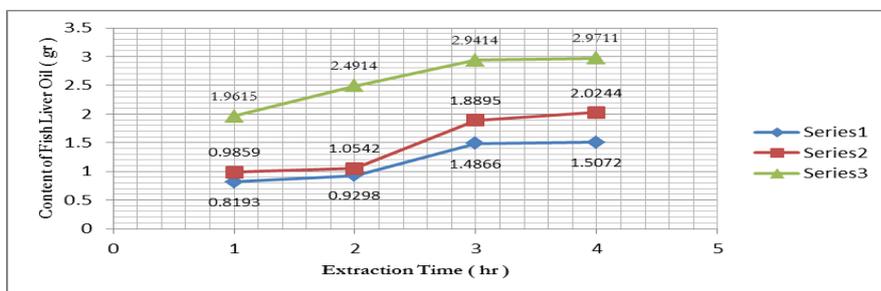
Also, according to Tables 1- 4, direct impact of time increase on fish liver oil extraction is clear. Thus, optimal temperature and time for achieving maximum salmon liver oil extraction is temperature 68 °C and 4 hour. Maximum extraction under these conditions is 2.9711 g. Fig 1 indicates diagram for salmon liver oil extraction (in terms of g) versus temperature (in terms of °C). Ascending trend of oil extraction with temperature increase is clearly observed in these diagrams for four specified times.



Series 1: Extraction Time is 1hr, Series 3: Extraction Time is 3hr, Series 2 :Extraction Time is 2hr, Series 4: Extraction Time is 4hr

Figure 1. Comparison of diagrams for changes in extracted oil content vs. extraction time during four times 1, 2, 3, and 4 h.

Fig 2 shows diagram for fish liver oil extraction in terms of duration of extraction process implementation by Soxhlet system for three operational temperatures (50, 60, and 68 °C) in comparative manner. Considering drawn diagrams, it is clear that fish liver oil extraction content is increased by time increase, and values obtained for times 3 h and 4 h are very close to each other, and it is shown that after f hours, no other change is observed in salmon liver oil extraction.



Series 1: diagram for Temperature 50°C, Series 2 : diagram for Temperature 60°C , Series 3 :diagram for Temperature 68°C

Figure 2. Comparison of diagrams for changes in extracted oil content vs. extraction time during three operational temperatures 50, 60, and 68 °

In order to investigate impact of volume of normal hexane solvent on oil extraction content, varying values of solvent (150, 200, and 300 ml) are used in optimal conditions of temperature and time as 68 °C and 4 hours. Results obtained from experiments are reported in Table 5.

Table 5: Impact of solvent on salmon liver oil extraction in optimal temperature (68 °C) and optimal time (4 h)

Percent of fish liver oil extraction	Average fish liver oil extraction (g)	Temperature (°C)	Time (h)
59.423%	2.9711	68	4
59.661%	2.9831	68	4
60.160%	3.0084	68	4

Results obtained from Fig 3 and Table 5 indicates increased oil extraction content from 2.9711 g for 150 ml solvent volume to 3.0084 g for 250 ml solvent volume. Of course, this increase is much smaller than other varying parameters (temperature, time) so that it can be stated it is better to use 150 ml solvent for saving matters, since not considerable change is observed in content of extracted oil.

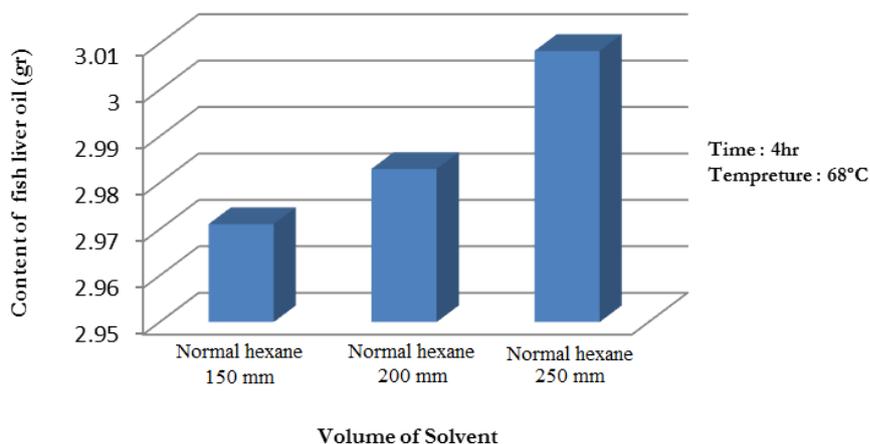


Figure 3. Diagram for changes on salmon liver oil extraction content vs. normal hexane solvent variable in optimal time and temperature

CONCLUSION

Studies in the current research suggest several important points which can be summarized as follows. Two important parameters including extraction operation temperature and operation time were effective on the content of salmon liver oil extraction, so that content of salmon liver oil extraction is increased by temperature increase during oil extraction process by Soxhlet system. Salmon liver is one of the wastes of this highly common used fish in our company. On the other, increasing time of extraction leads to considerable increase in content of oil extraction from lowest amount of 0.8193 g to highest amount of 2.9711 g. of course, it should be noted increasing the time more than 4 hours had no additional impact on oil extraction content. Optimal temperature and time parameters leading to maximum oil extraction is 4 hours and 68 °C temperature. That is, 2.9711 g content of oil extraction was obtained for this temperature and time by Soxhlet system. Results obtained from investigation of third parameter, i.e. n-hexane solvent showed impact of solvent on application of varying values of solvent, i.e. 150, 200, and 250 ml, so that it was observed salmon liver oil extraction content was increased by increase in normal hexane solvent content. It suggest the fact that the solvent also has direct impact on oil extraction content, and fish liver oil extraction content was increased from 2.9711 g for 150 ml solvent to 3.0084 g for 250 ml solvent; though, impact of increasing solvent content on oil extraction content was less than two other variables (temperature and time). Thus, it can be concluded that maximum fish liver oil extraction content is observed under optimal conditions of 68 °C and 4 hours and normal hexane solvent volume of 250 ml.

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