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RESEARCH ARTICLE

Effects of heat treatment on fish oil extraction process using a single screw expeller

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ABSTRACT

Effects of heating temperature and duration on yield and quality (free fatty acids value, saponification value, para-anisidine value and peroxide value) of oil extracted from fish using a single screw expeller was studied. Heating temperature and duration were 60, 70, 80 and 90 °C and 5, 10, 15 and 20 minutes respectively. All data collected were subjected to analysis of variance (ANOVA) to test for significant effects at 95 % confidence limit. When significant difference was observed, treatment means were separated using the F-LSD. The oil yield ranged between 17.2 % and 21.6 %, free fatty acid values (2.2–4.5 %), saponification values (134-189 mgKOH/g), para-anisidine values (7.3-12.2 mEq/kg) and peroxide values (8.1–11.7 $mEqO_2$/kg). The treatments have significant effects on oil yield, free fatty acid value, saponification value, para-anisidine value and peroxide value or at $p<0.05$. The mean separation also shows significant effects on oil yield, free fatty acid value, saponification value, para-anisidine value and peroxide value at $p<0.05$. Optimum temperature and duration of heating were 90 °C and 15 minutes respectively. This combination gave 21.6% oil yield, 2.5 % free fatty acid value, 136 mgKOH/g saponification value, 10.8 mEq/kg para-anisidine value and 8.3 $mEqO_2$/kg peroxide values.

Keywords: Oil extraction; fish oil; screw expeller; process parameters

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INTRODUCTION

Fish is one of the most popular food items for human consumption throughout the world. Fish oils have gained much more importance because of the presence of health beneficial omega-3 fatty acids in them. These polyunsaturated fatty acids (PUFA) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) play a crucial role in the prevention of atherosclesis, heart attack, depression, stroke, diabetes, obesity, premature ageing, hypertension, cancer and improve the vision power and memory (Adeniyi, 2006; Hee-Guk et al., 2008).

Fish oil is the lipid fraction extracted from fish and fish by products. Generally, fish oils are more complex than land-animal oils or vegetable oils due to long – chain unsaturated fatty acids. Fish oils are unique in the variety of fatty acids of which they composed and their degree of un-saturation (Fournier et al., 2007; Zhong et al., 2007; Valeria et al., 2010). Refined fish oils are rich in polyunsaturated fatty acids of the linolenic acid family. Current medical research suggests that these fatty acids might have a unique role to play in prevention of coronary artery disease and the growth of different types of cancers. The oil is industrially used in leather tanning, production of soap and glycerol, and other

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products. Fish meal is a ground solid product that is obtained by removing most of the water and some or all oil from fish or fish by-products and is mainly used for the production of animal feeds.

Fish oil extraction processes can be classified into three categories: physical, biological and chemical. Physical extraction processes include homogenizing, heating, pressing and filtering, also regarded as wet rendering (Thitiphan and Waranya, 2015). Biological processes include enzymatic oil extractions and silage production through the use of enzymes from fish viscera residue (autolysis) or enzymes from other sources (hydrolysis). Chemical solvent extraction is another well-established process to extract fish oil using organic solvents, however, the use of toxic solvents results in protein denaturization and loss of functional properties (Sahena et al., 2010; Maqsood et al., 2012). Supercritical fluid extraction technology (SCFE) has also been proposed in the extraction of compounds from natural sources including oil recovery from seeds/biomass, raw fish and/or fish by-products. SC- CO_2 for oil recovery is an attractive option as it is a non-toxic, nonflammable, inexpensive and clean solvent.

Wu and Peter (2008) stated that there are physical factors that can affect the production of fish oil, one of which is processing temperature. High temperature can cause the oxidation process resulting in fat breakdown. Proteins usually undergo irreversible denaturation when heated at 90-100 °C. Denatured proteins forming a dense structure can cause inhibition of oil release. Sugeng and Nurjanah (2015) showed that the yield of extracted fish oil increased from 25 °C to 80 °C, but decreased at extraction temperature of 90 °C. According to Sugeng and Nurjanah (2015), the optimum temperature for wet rendering was 80 °C.

Extraction and purification of the lipids by conventional methods, such as hexane extraction, vacuum distillation, or conventional crystallization have the disadvantages of requiring high temperature processing which results in decomposition or degradation of the thermally labile compounds and/or employing toxic solvents having adverse health effects (Maqsood et al., 2012). Therefore, various research efforts are currently focusing on developments in the field of oil extraction and purification technologies. The demands on these processing technologies for extracting and purifying the fish oil are that they are eco-friendly and able to provide high oil yields and to minimize the loss of nutrients and provide a high quality oil (Maqsood et al., 2012).

Edible applications of fish oils

Their nutritional and physical properties have made hardened fish oils attractive constituents in diets for man. Hardened fish oil is used almost entirely in margarines and shortenings. Margarines prepared from hardened vegetable oil sometimes recrystallize on storage. This makes the margarine crumbly and hard. Because fish oils have a widely varied chain length, margarines prepared from them have an excellent plastic consistency. Shortening and bakery margarines have properties different from those of table margarines. Refined fish oils are rich in polyunsaturated fatty acids of the linolenic acid family. Current medical research suggests that these fatty acids might have a unique role to play in prevention of coronary artery disease and the growth of different types of cancers.

Technical applications of fish oils

The highly unsaturated properties make the oils (and particularly their highly unsaturated fractions) suitable for a number of technical applications, particularly as drying oils and varnishes. The saturated fatty acid fraction is a disadvantage for these purposes and must be reduced. Several specialized processes for this reduction are available. Fish oils are a significant source for the production of fatty acids with a wide spectrum of chain lengths. From these acids are produced several types of metallic soaps, some of which are used in lubricating greases while others are used as waterproofing agents. Small quantities of fatty; acids are used pharmaceutically and medicinally, and for scientific research purposes.

MATERIALS AND METHODS

Samples preparation and heat treatment

For this research work, oily fish species (Atlantic mackerel) were obtained fresh from the market in Makurdi. Prior to analysis, the internal organs were removed and the fish was washed to remove the residual blood. The fish was cut into small pieces. The material is heated using a microwave oven to coagulate the proteins and disrupts the cell membranes thus allowing the leakage out of bound water and oil.

The levels of variables were decided partly from a review of relevant literature but mainly from preliminary investigations. Heating temperature were 60, 70, 80 and 90 °C while 5, 10, 15 and 20 minutes were heating duration. The treatment combination is as shown in Table 1. About 6000 g of the fish was used as sample material in each experiment. The sample was heated in a microwave oven at the desired heating temperature level. A thermometer was used to verify the oven temperature.

Oil extraction by mechanical press

The literature reveals that mechanical screw press oil extraction is widely used in commercial and industrial edible oil extraction, as a result of safety and simplicity of the whole process. (Singh and Bargale, 2000; Uquiche et al., 2008; Stanisavljevic et al., 2009).

The oil extraction was done mechanically with an oil expeller press. The expeller powered by a 5hp electric motor was set into operation and known weights (6000 g) of each prepared sample were fed into the machine through the feeding hopper. The interrupted helical screw drum conveyed, crushed, squeezed and pressed the fishes in order to extract the oil. The oil and water phases (containing water-soluble proteins as well) are separated from the solid phase (press cake). The fluid extracted and the press cake were collected and weighed separately. Figures 1 is the isometric drawing of the expeller press.

Figure 1: Isometric drawing of the expeller press

Clarification was done to separate the oil from its entrained impurities. The fluid extracted out of the press is a mixture of fish oil, water, cell debris, and non-oily solids. The fluid was allowed to stand undisturbed to settle by gravity so that the oil, being lighter than water, will separate and rise to the top. The clear oil was decanted into a reception container, sieved and heated to remove moisture in the oil. Oil extract from fish was placed into a bottle, sealed, and stored away

from sunlight at a temperature under 5 °C until further analysis. Figure 2 is a photograph of the extracted fish oil in plastic bottles.

Figure 2: The extracted fish oil in plastic bottles

Method of evaluation and statistical analysis

From the values obtained, the oil yield was calculated. The extraction performance of the machine was evaluated by expressing the oil extracted as a percentage of the total oil content of the fish samples. From the values obtained, oil yield was calculated according to Olaniyan and Oje (2007, 2011), and Adesoji et al (2013) as:

$O_Y = \frac{100\,W_{OE}}{W_{FE}+W_{RC}}$ % Where; W_{OE} = Weight of oil extracted, W_{FE} = Weight of fish extract, W_{RC} = Weight of residual cake.

Statistical analysis

The experimental design for the statistical analysis follows a two-treatment effect (heating temperature and heating time) in a split-plot factorial design with Completely Randomized Design (CRD) involving a two-way classification with three observations (replications) per experimental unit. The experimental unit comprises two factors; four heating temperatures (60, 70, 80 and 90 °C) in each of the four levels of the heating times (5, 10, 15 and 20 minutes) giving a sixteen treatment combinations for the experiment as heating temperature versus heating time. The heating temperature in the combination forms the levels of factor 'A' while the heating time forms the levels of factor 'B'. All data collected were subjected to analysis of variance (ANOVA) to test for significant effects at 95 % confidence limit using the procedure recommended by Steel and Torrie (1980). When significant difference was observed, treatment means were separated using the F-LSD.

Analysis of fish oil quality

The physicochemical properties of the oil are determined to assess its quality and purity. Four oil quality indices was used to determine the quality of the extracted oil. They are the free fatty acid content, saponification value, para-anisidine value and peroxide value. The level of free fatty acids (FFA), saponification value (SV), para-anisidine value (pAV) and peroxide value (PV) were determined by titration according to the Official Method of Analysis of the Association of Analytical Chemist (AOAC, 2005).

RESULTS AND DISCUSSION

Effects of heat treatment on oil yield

The result of the effect of heat treatment on mean fish oil yield (%) is shown in table 1. The individual and interactive effects of such process parameters such as heating temperature and heating time on the oil yield were evaluated. The oil content of the seed from the proximate analysis is 26.8 %. The analysis of variance result is shown in table 2. This table shows that the individual and interactive effects were significant at $p \leq 0.05$. The F-values also indicated the order of significance of the process parameters giving heating temperature as the most important variable that affected the oil yield followed by heating time.

A 2-tailed F-LSD test at 5% level of significance shows that the differences between the heating temperatures (θ) and heating times (T) treatment combinations are statistically significant except the differences between the heating temperatures (θ) and heating times (T) treatment combinations of θ_4T_4 and θ_2T_3, θ_4T_4 and θ_3T_1, θ_4T_4 and θ_3T_4, θ_3T_4 and θ_1T_3, θ_3T_4 and θ_2T_2, θ_3T_4 and θ_2T_3, θ_3T_4 and θ_3T_1, θ_3T_1 and θ_1T_3, θ_3T_1 and θ_2T_2, θ_3T_1 and θ_2T_3, θ_2T_4 and θ_1T_2, θ_2T_2 and θ_1T_2, θ_2T_2 and θ_1T_3, θ_2T_1 and θ_1T_1, θ_2T_1 and θ_1T_4, θ_1T_4 and θ_1T_1, θ_1T_3 and θ_1T_2 which are not statistically significant (Table 1).

Figure 3 shows the effect of heating time and heating temperature on oil yield. It was observed from Figure 3 that the oil yield increased with increasing heating temperatures from 5 to 15 heating times and decreased at 20 minutes heating time. The best oil yield of 21.6 % was obtained for the conditions of 90 ^{o}C heating temperature and 15 minutes heating time. The lowest oil yield of 17.2 % was obtained for the conditions of 60 ^{o}C and 5 minutes heating time. The oil yield increases towards the centre of the plot and moves away from the centre as heating time is further increased resulting to reduction in oil yield. This suggests the centre area as boundary were optimization of oil yield could be obtained. This temperature trend is in agreement with previous works which attribute this behaviour of oilseed to the fact that heat coagulates the protein and reduces the viscosity of the oil thereby facilitating oil expression process as moisture reduction takes place simultaneously. At higher temperature, prolonged heat treatment causes a substantial moisture loss leading to hardening of oil seed sample which best explains the reason behind the reduction in yield at higher temperature (Alonge et al., 2003; Bamgboye and Adejumo, 2011). This observation conforms to findings on previous works carried out on dika nut, groundnut, and shea kernel (Olaniyan and Oje, 2007; Abidakun et al., 2012; Olajide et al., 2014).

Table 1: Effect of heat treatment on mean fish oil yield (%)

Heating Time, T	Heating Temperature, θ (^{o}C)			
	60	70	80	90
5	17.2	17.4	18.7	19.3
10	18.1	18.4	19.8	20.5
15	18.4	18.9	21.0	21.6
20	17.3	18.0	18.6	18.9

$F\text{-}LSD_{0.05} = 0.374$

Table 2: Analysis of variance of the effect of heat treatment on oil yield (%)

Sources of Variation	DF	SS	MS	F	P
Heating Temperature, θ (^{0}C)	3	598.05	199.349	284.82*	2.90
Heating Time, T (mins)	3	384.64	128.213	183.19*	2.90
Interaction (θ × T)	9	57.99	6.443	9.21*	2.19
Error	32	22.40	0.700		

Total	47	1063.7
* - Significant		

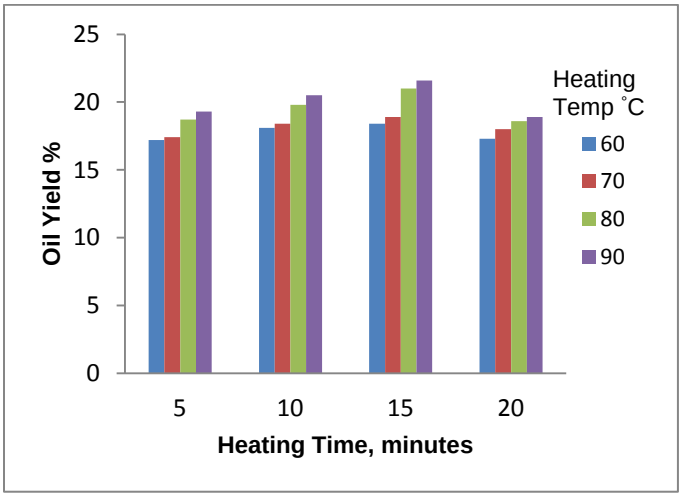

Figure 3: The interaction effect of heating time and temperature on oil yield

Effects of heat treatment on fish oil quality

In order to determine the stability and quality of fish oil extracts, some quality assessment was conducted. It was observed that all the results obtained were tolerable to the standard values. Table 3 shows the oil quality analysis results.

i. Free fatty acid (FFA) value

The results showed decrease in FFA with increase in both heating temperature and duration. The higher extraction temperature was resulting in fish oil with lower FFA value. FFA values decreased accordingly with increasing extraction temperatures. The highest FFA value of 4.5 % was obtained at 60 ^{0}C heating temperature and 5 minutes heating time while the lowest FFA value of 2.2 % was obtain at 90 ^{0}C heating temperature and 20 minutes heating time. This suggests that lipase, which initiates rapid hydrolytic deterioration in oil, is deactivated by heat treatment. A change in free fatty acid content of vegetable oil depends on time and temperature of heating oil bearing materials among other factors (Akinoso et al. 2006).

Khoddami et al. (2009) informed that increasing extraction temperature can cause faster formation of free radicals and free fatty acids. High extraction temperature could trigger the onset of FFA impairment, in which free fatty acids were degraded into volatile components (Weber et al. 2007). Furthermore, high extraction temperature induces denaturation of lipase in the cooked samples. It will prevent the release of free fatty acid component (Weber et al. 2007). Chantachun et al. (2000) also found in his study that low value of FFA obtained from tuna oil extracted using at 95 °C.

Differences of extraction temperature and duration affected the free fatty acid value in fish oil ($P<0.05$). FFA content of the overall combinations of extraction temperature and period was in the range of 2.2 to 4.5 %. Standard FFA value in fish oil according to the International Association of Fish Meal and Oil Manufacturers (IFOMA) (1981) is in the range of 1-7% oleic acid. FFA values for all fish oil obtained in this study were within the IFOMA standard.

ii. Saponification value (SV)

Saponification value is the amount of alkali necessary to saponify a given quantity of fat/oil. It is expressed as the milligrams of potassium hydroxide required to saponify 1 g of the sample. As all fatty acids "bind" one molecule of potassium hydroxide, it can be used to measure the average molecular weight of the triglycerides of the sample. The smaller the saponification value, the longer the average fatty acid chain length. Literature values show a considerable range for the saponification values, but most fall between 132 and 207.5 mg KOH/g (Honfo et al., 2014). Oil samples with higher saponification value (above 195) are not attractive as raw material for the edible oil, because of cost of refining. However, they are best used as raw materials in the soap industry because the greater the saponification value, the better the soap making ability of the oil.

Results of the experiment showed that saponification values decreased with increase in heating temperature and heating time. The highest saponification value of 189 mgKOH/g was obtained at 60 ^{0}C heating temperature and 5 minutes heating time while the lowest saponification value of 134 mgKOH/g was obtain at 90 ^{0}C heating temperature and 20 minutes heating time. Adejumo et al. (2013) also had the similar trend of decreasing saponification value with increase in temperature. This is because temperature is one way to eliminate the compounds of other impurities and allow the natural process of saponification. The higher value suggests that the oil contain high molecular weight fatty acids with low level of impurities and can be used in soap making industry. This shows that the quality of the oil decreases due to the process of oxidation and hydrolysis (Soetaredjo et al., 2008).

iii. Para-anisidine value (pAV)

Hydroperoxide decomposition will produce secondary oxidation products such as aldehydes, ketones, acids, alcohols, hydroxy component, lactones, hydrocarbons, dienal, epoxide monomer and polymer compounds, etc. Secondary oxidation product is measured as p-anisidine value (Okullo et al., 2010).

From the results, extraction period affected the p-anisidine value. The longer extraction time, the higher p-anisidine value will be. The highest p-anisidine value of 12.2 mEq/kg was obtained in oil extracted at 90 °C heating temperature and 20 minutes heating time while the lowest p-anisidine value of 7.3 mEq/kg was obtained in oil extracted at 60 °C heating temperature and 5 minutes heating time. Longer time of extraction period will bring about the maximum decomposition of hydroperoxides leading to generate secondary oxidation products.

Extraction temperature and period significantly ($P < 0.05$) affected p-anisidine value of the oil extracted. According to IFOS (2011), p-anisidine value should be less than 15 meq/kg. Overall p-anisidine values of fish oil obtained in this study was still in the range of standard set by the IFOS.

iv. Peroxide value (PV)

Peroxide formation occurs during primary oxidation. Fish oil contains high number of unsaturated fatty acid, so it is easily oxidized to become rancid. Rancidity protracted will form peroxides and decrease the quality of the fish. The unpleasant rancid odor due to the formation of aldehydes or ketones as derived products of hydroperoxides. The oil/fat breakdown led to the formation of free radicals caused by light, heat, fat peroxide, heavy metals, hematin, hemoglobin, and several other causes. Free radicals can react with oxygen to form peroxide compounds which ultimately affect the physical and chemical properties of the oil. Peroxide value is a trace of oxidation and rancidity. The lower the peroxide value, the better the oil quality. It is known that factors such as temperature, light, moisture, metals, and oxygen affect rate of oxidation. This is a major cause of their deterioration (Okullo et al., 2010).

From the experimental results, the peroxide values decreased with increasing heating temperature and heating time. The highest peroxide value of 11.7 $mEqO_2$/kg was obtained at 60 ^{0}C heating temperature and 5 minutes heating time while the lowest peroxide value of 8.1 $mEqO_2$/kg was obtain at 90 ^{0}C heating temperature and 20 minutes heating time. Heat affects the initiation of autooxidation process. High temperature exposure to the sample will be resulting in high content of peroxides. Results showed that the decreasing of peroxide value occured when extraction was carried out at 90 °C probably also due to fewer oxygen dissolved in the material. If the water temperature raised, dissolved oxygen will decrease. Temperature increase can cause water molecules move more quickly, thus it can destroy the hexagonal structure and release the trapped oxygen. The critical point will occur at a temperature of 100 °C, when it is no longer dissolved oxygen (Okullo et al., 2010). Free radicals can trigger the formation of peroxides when it reacts with oxygen. Extraction temperature at 90 °C could make the material lost much dissolved oxygen.

Table 3: Analysis of Fish Oil Quality

Heating Time (minutes)	Heating Temperature (°C)	Free Fatty Acid Value (FFA) %	Saponification Value (SV) mgKOH/g	Para-anisidine (pAV) mEq/kg	Peroxide Value (PV) $mEqO_2$/kg
5	60	4.5	189	7.3	11.7
	70	3.9	187	7.7	10.5
	80	3.5	183	8.0	10.1
	90	3.2	180	8.5	9.9
10	60	3.9	179	8.6	10.2
	70	3.6	174	8.9	10.0
	80	3.3	169	9.2	9.5
	90	3.0	162	9.7	9.0
15	60	3.7	157	9.0	9.2
	70	3.4	150	9.4	8.8
	80	3.0	144	10.4	8.5
	90	2.5	136	10.8	8.3
20	60	3.2	155	10.3	8.7
	70	3.1	146	11.4	8.4
	80	2.5	140	11.8	8.3
	90	2.2	134	12.2	8.1

Extraction temperature and period affect peroxide value significantly ($P < 0.05$). The higher temperatures, the lower peroxide value will be. It means the peroxide value decreased with the elevating of temperature. According to IFOS (2011), Peroxide value should not be exceeding 3.75 meq/kg. Peroxide value of all extracted fish oil have been higher than IFOS. Bimbo (1998) has reported that peroxide value (PV) of crude fish oil was between 3 and 20 meq/kg. In this study, the PV was found to be between 8.1 and 11.7 meq/kg, which is well below acceptable limit of 20 meq O2/kg oil. This indicated that the fish oil extracted had low lipid oxidation rate.

CONCLUSION

This study on effect of process parameters on oil yield and quality from fish using mechanical screw press revealed that the most important variable is heating temperature followed by heating duration. Heating temperature content had the most significant effect while heating time had the least effect on oil yield. The maximum oil yield of 21.6 % was obtained for 90 ^{0}C heating temperature and 15 minutes heating time. This implies that these process parameters must

be controlled to effectively extract oil fish. Hence, this knowledge is a great guide to researchers and designers for future work on fish oil production. The result obtained also shows that this process (mechanical extraction) is a suitable method for extracting fish oil because of its high yield and high oil purity, both in large or small quantity.

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