

**Optimization of the traditional process of oil extraction from sardine viscera (*Sardina pilchardus*) in the Far North Region-Cameroon by the Response Surface Methodology**

**ABSTRACT**

**Aims:** *Sardina pilchardus* is a fish highly found in rivers and lakes in the Far-North region of Cameroon. This work aimed at optimizing the traditional extraction parameters in order to improve the quality and yield of oil.

**Methodology:** A central composite design was used to study the effect of processing (cooking time, viscera/water ratio and settling time) on the extraction yield and quality of oil. The yield and quality of the oil obtained were compared to the traditional extraction method.

**Results:** It appears that the cooking and the settling times increased the extraction yield. The cooking time, the viscera/water ratio and their interactions reduced the acid value. The viscera/water ratio contributed to the reduction of the peroxide value. **Optimal condition was found to be at cooking time of 17.02min, viscera/water ratio of 100:152.86g/mL, settling time of 95.59 min, at the temperature of 95°C.** The optimized processing conditions improved the extraction yield by 10 to 25%, an increase of 2.3 times compared to the traditional process and preserved the quality of the oil (acid value of 1.35 mg KOH /g oil and peroxide value of 1.20 meq O<sub>2</sub>/kg oil).

**Conclusion:** The relationship between the actual and predicted data indicates that the response model was adequate to reflect the expected optimization. This will therefore help fish oil producers in the Far North region of Cameroon.

**Keywords:** Fish, *Sardina pilchardus*, Viscera, Oil, Traditional process, Optimization

## 1. INTRODUCTION

Fisheries and aquaculture represent one of the most important sectors for protein supply according to the food and agriculture organization [1]. Statistics presented by this organization show that world fish production has increased from 163 million tons in 2010 to around 177 million tons in 2020 [1]. FAO [2] reported that not all of the quantity of fish produced each year is consumed. About 50% of the total fish mass is generated as by-products or waste materials during the fish processing operations. Hence, the increase in fish production in the world leads to the increase of by-product generation, including viscera, head, scales and bones. As a result, this huge quantity of by-products, there is a need of looking for ways of valorisation. Thus, the viscera are used by certain local populations as a potential source of fish oil [3, 4]. Fish oil is known to be rich in long chain polyunsaturated fatty acids mainly composed of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) recommended for nutrition and useful for human health [5].

Fish oil is usually obtained from whole fish or fish waste by chemical treatments, or by cooking and pressing using conventional extraction methods such as Soxhlet extraction [6], steam entrainment [7], or slightly more advanced so-called green methods such as microwave-assisted [8], ultrasound-assisted [9], supercritical fluid [10, 11] and enzymatic extraction [12]. The above methods have many drawbacks, particularly the high investment cost and operating pressure which can deteriorate the oil quality. The use of solvents is most often expensive, requires a lot of energy to separate the oil from the solvents used, the faster degradation of lipids due to high temperatures in the presence of solvent. For some raw materials like viscera, the extraction by pressing is not adequate. All these prevents their implementation in small or moderate scale production systems[13, 14].

Furthermore, the conventional and green methods are still difficult to implement in most Africa countries and in Cameroon in particular which is facing energy crisis. The traditional extraction methods are used as alternatives to obtain fish oil and present the advantage of being affordable in term of cost [15, 16, 17]. Due to their proliferation in the far Nord region of Cameroon, sardine is traditionally processed there and their viscera are used for oil

extraction. Also, the consumption of fish oil has become a source of income for households, beyond its production for domestic requirements. This fish oil is highly demanded locally and even in neighboring countries [4]. Based on our observations on the field (far Nord region) and preliminary analyses, the extraction yield oscillated from 8-12%, the quality indices which are the acid and peroxide value vary between 5.9-7.9 mg KOH/g oil and 7.5-9.5 meq/kg oil respectively. These preliminary results arise from the fact that the extraction processes vary from a producer to the other and the variability of unit operations affects the quality and yield of the fish oil produced [4]. Several authors have solved the fish oil yield extraction problems through optimization, using Response Surface Methods (RSM) [18, 19], which appears as a tool to address the glitches faced by the rural communities of the Far North region of Cameroon.

The aim of this work was to optimize the traditional process of extracting oil from sardine viscera (*S. pilchardus*) carried out in the localities of the Far North region of Cameroon by applying the RSM in order to improve the yield and guarantee the quality of locally produced sardine oils.

## **2. MATERIALS AND METHODS**

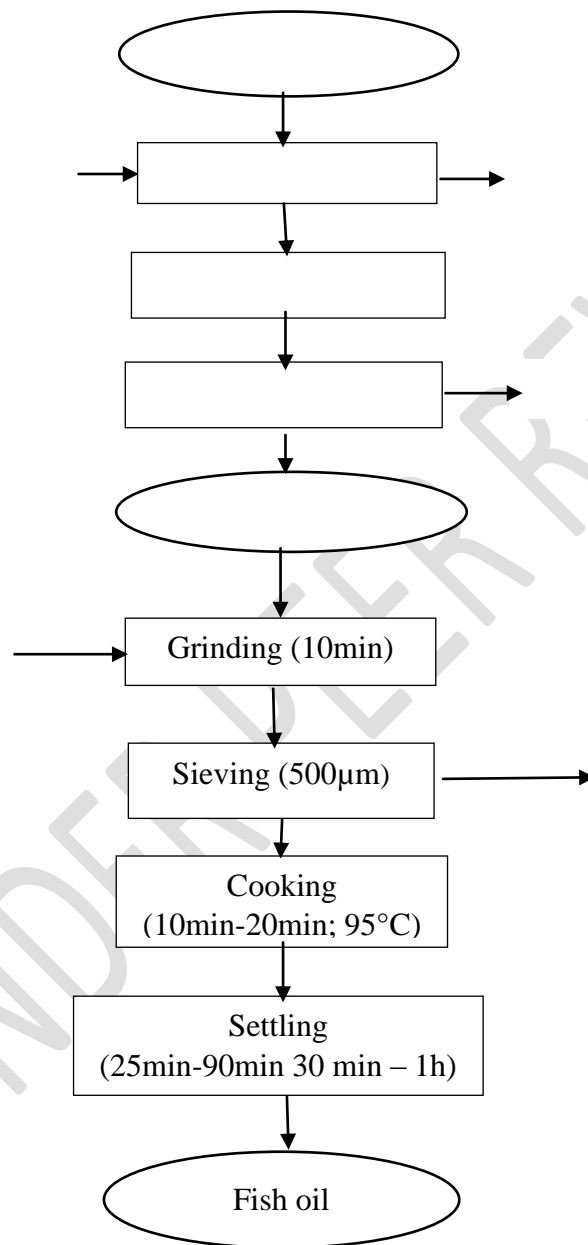
### **2.1 Materials**

The fresh sardines were purchased on the shores of Lake Maga located in the Far North region of the Mayo Danay Division (latitude: 10°48'9.41" North, longitude: 14°57'6.81" East). Batches of approximately 5 to 10 kg were purchased from each fisher. The samples were then kept in coolers containing ice cubes and then transported to the RESH laboratory at the University of Ngaoundere. The temperature inside the cooler at the starting of the journey and the arrival was around -5°C to 0°C.

### **2.2 Sample preparation and extraction procedure of sardine oil from viscera**

The fish upon arrival at the laboratory was immediately washed with large quantities of fresh water, destemmed and eviscerated. The viscera obtained were used for oil extraction. The wet rendering extraction plan was applied in this study as shown in the schematic diagram

(Figure 1). The viscera were then macerated in cold water for 5 to 10 minutes and then left to stand for settling for 30 minutes to 1 hour. After settling, the viscera are cooked in a pot with water of proportion (100:100(g/ml)). Cooking lasted 10 to 20min at a temperature of 95°C. Cooking ended when the white floating layer turns light yellow or golden yellow with the lumps disappearing. During cooking, the pot was not closed.



**Fig. 1.**Flow diagram of sardine viscera oil's extraction

At the end of each extraction process, the percentage of oil extracted was computed as follow:

## 2.3. Experimental Design and Statistical Analysis

### 2.3.1. Determination of the factor ranges

Prior to the optimization of oil extraction from viscera of sardine (*S. pilchardus*), a one variable at a time design was performed in order to determine the range of each selected factors. The experimental factors namely cooking time ( $X_1$ ), viscera/water ratio ( $X_2$ ), and settling time ( $X_3$ ) are presented in table 1 in their real and coded values.

**Table 1.** Independent variables used to optimize the extraction process.

Variables	Units	Natural values		Coded values	
		Low	High	Low	High
$X_1$	Min	10	20	-1	+1
$X_2$	g/ml	100:100	100:200	-1	+1
$X_3$	Min	25	85	-1	+1

### 2.3.2. Central Composite Design (CCD)

In this study, a second-order polynomial model (equation 1) was used through Central Composite Design to determine the effect of three the variables on the extraction yield of fish oil from the viscera of sardine fish as well as the quality of the final product. The factors evaluated were those which are critical for the extraction process.

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 \quad (1)$$

where  $Y$  values were the responses (dependent variables);  $X_1$ ,  $X_2$ , et  $X_3$  the factors (coded independent variables);  $a_0$  the value of the coefficient at the center point;  $a_1$ ,  $a_2$ ,  $a_3$  the linear terms;  $a_{12}$ ,  $a_{13}$ ,  $a_{23}$  the interactive terms;  $a_{11}$ ,  $a_{22}$ ,  $a_{33}$  the quadratic terms.

The extraction yield, acid and peroxide values were the three responses monitored throughout the experimentations. The experimental design is given in table 2 and the three

central points which are one of the main characteristics of the central composite design used are the runs 15, 16, and 17 respectively. A total of 17 experiments were done as shown in the experimental matrix.

**Table 2.** Experimental matrix for the extraction process.

Run	Coded Independent variables		
	Cooking time ( $X_1$ )	Viscera/water ratio ( $X_2$ )	Settling ( $X_3$ )
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-1.35313	0	0
10	1.35313	0	0
11	0	-1.35313	0
12	0	1.35313	0
13	0	0	-1.35313
14	0	0	1.35313
15	0	0	0
16	0	0	0
17	0	0	0

The average absolute deviation (AAD) of a set of data, the bias factor ( $Bf$ ), the accuracy factor ( $Af$ ) and  $R^2$  were calculated and compared to standard values. For a model to be valid the AAD should be close to zero while the bias factor and accuracy factor should be between 0.75 and 1.25, and  $R^2$  value should be closed to 1 [20]. The computation was done using the following formulas:

$$R^2 = \frac{\sum(Y_{ical} - \bar{Y})^2}{\sum(Y_{iexp} - \bar{Y})^2}$$

$$AAD = \frac{1}{n} \sum_{i=1}^n \left( \frac{|Y_{exp} - Y_{cal}|}{Y_{exp}} \right)$$

$$Bf = 10^{\frac{1}{n} \sum_{i=1}^n \log\left(\frac{Y_{cal}}{Y_{exp}}\right)}$$

## 2.4 Physicochemical parameters of extracted oils

The physicochemical parameters of fish oil extracted such as water content, acid, peroxide, saponification values were determined according to the method described in A.O.C.S [21]. Relative density and refraction index and iodine value were determined according to the method described in AFNOR [22].

## 2.5 Extraction of total lipids

The extraction of total lipids was done by the method of Bligh and Dyer[23]. In this method, the lipids are extracted by a ternary mixture of chloroform/methanol/water in proportion 1/2/1 (V/V/V) for 1 g of sample. The percentage of oil extracted was computed as follow:

$$\text{Yield of fish oil (\%)} = \frac{\text{Weight viscera used (g)}}{\text{Weight viscera used (g)}}$$

## 2.6 Optimization of oil extraction from sardine's viscera

The optimization of the extraction parameters was aimed to maximizing the extraction yield while preserving the qualities of the oil. To meet up with these goals, the criteria set for each response were defined as minimizing the Peroxide value, the Acid value and maximizing the extraction yield based on the example of [18].

## 2.7 Statistical analyses

The methodology of the statistical analyses of the extraction yield, acid value and peroxide value was obtained using Design Expert software package. The software package was also used to generate the experimental design (table 2) and to carry out statistical analysis (ANOVA) of the models and to plot the curves.

# 3. RESULTS AND DISCUSSION

## 3.1. Model Fitting

A total of seventeen experiments including three center points were carried out in triplicates to evaluate the effect of cooking time (Ct), viscera/water ratio (VWr) and Settling time (St) on the Extraction yield (Ey), Acid value (Av) and Peroxide value (PV). Data recorded are given

in table 3 and analyzed through multi-regression analysis. The results revealed that linear and interactive (2FI) terms have lower p-values when compared with the quadratic terms.

**Table 3.** Experimental matrix with different responses

Run	Ct (X <sub>1</sub> )	VWr (X <sub>2</sub> )	St (X <sub>3</sub> )	OEy (Y <sub>1</sub> )	OEyP (Y <sub>1</sub> )	Av (Y <sub>2</sub> )	AvP (Y <sub>2</sub> )	Pv (Y <sub>3</sub> )	PvP (Y <sub>3</sub> )
1	10	100:100	25	8.8	8.3	1.66	1.68	2.1	2.4
2	20	100:100	25	20.5	20.7	23.66	23.69	3.2	3.5
3	10	100:200	25	0.5	0.8	1.43	1.47	1.9	1.4
4	20	100:200	25	0.8	1.2	1.94	1.96	2.6	2.8
5	10	100:100	85	12.9	13.1	1.66	1.68	2.1	2.5
6	20	100:100	85	34.8	35.4	23.66	23.69	3.2	3.5
7	10	100:200	85	1.5	1.8	1.43	1.47	1.9	1.4
8	20	100:200	85	11.9	12.4	0.95	1.00	1.2	1.6
9	8.23	100:150	55	0.3	0.5	1.94	1.98	2.6	2.8
10	21.76	100:150	55	2.9	3.1	1.74	1.77	2.2	2.5
11	15	100:82.34	55	21.7	21.9	22.67	22.70	3.1	3.6
12	15	100: 217.65	55	0.1	0.3	0.95	1.00	1.2	1.6
13	15	100:150	14.40	2.5	2.8	1.09	1.12	1.5	1.7
14	15	100:150	95.59	10.8	11.1	1.09	1.12	1.5	1.7
15	15	100:150	55	8.6	8.8	1.09	1.12	1.5	1.7
16	15	100:150	55	8.7	8.9	1.09	1.12	1.5	1.7
17	15	100:150	55	8.2	8.4	1.09	1.12	1.5	1.7

Ct: Cooking time, VWr: Viscera/Water ratio, St: Settling time, OEy: Oil Extraction yield, OEyP: Oil Extraction yield Predicted, Av: Acid value, AvP: Acid value Predicted, Pv: Peroxide value, PvP: Peroxide value Predicted.

A second-order polynomial equations were developed (equation 2, equation 3 and equation 4) to understand the interactions between the processing variables. The positive signs of X<sub>1</sub> and X<sub>3</sub> shows that they contribute positively to improve the yield of fish oil while the negative sign of X<sub>2</sub> shows that the proportions of viscera/water could have a negative contribution at a certain extent. All the same, the sign of the quadratic coefficients give the nature of the contributions of various factors on the response [18].

$$Y_1 = 4,79 + 3,24 X_1 - 6,99 X_2 + 2,72 X_3 - 1,01 X_1^2 + 4,07 X_2^2 + 1,75 X_3^2 - 1,61 X_1 * X_2 + 1,29 X_1 * X_2 + 0,46 X_2 * X_3 \quad (2)$$

$$Y_2 = 1,18 + 3,75 X_1 - 6,37 X_2 - 0,08 X_3 + 0,30 X_1^2 + 5,74 X_2^2 - 0,11 X_3^2 - 5,50 X_1 * X_2 - 0,12 X_1 * X_2 - 0,12 X_2 * X_3 \quad (3)$$

$$Y_3 = 1,52 + 0,14 X_1 - 0,48 X_2 - 0,12 X_3 + 0,47 X_1^2 + 0,33 X_2^2 - 0,03 X_3^2 - 0,28 X_1 * X_2 - 0,18 X_1 * X_3 - 0,18 X_2 * X_3 \quad (4)$$

Where  $Y_1$ ,  $Y_2$  and  $Y_3$  are respectively the extraction yield, acid value, and peroxide value.  $X_1$ ,  $X_2$ , and  $X_3$  stand for cooking time, viscera mass/water volume ratio and settling time respectively.

### 3.2 Analysis of variance (ANOVA) for the regression models

The adequacy of the models was tested using ANOVA. Table 4 presents the regression coefficients of the response surface models and statistical analysis of each response. The effect of a parameter on the response is considered significant only if the p-values are less than 0.05 [18, 20, 24]. Both regression models have p-values less than 0.05. All the models are valid and can then be used for exploratory analysis of the factors.

The experimental data for each measured variable were fitted to a quadratic model. Table 4 shows the polynomial coefficients for each surface response model calculated by multiple regression as well as their associated P-value. It was observed that yield extraction was dependent on the linear effect of cooking time, viscera/water ratio, settling time ( $P < 0.05$ ). This shows that the three variables studied had effect on the fish oil yield and on the acid value of the oils obtained. However, the settling time exerted no significant effect upon the AV, while cooking time and settling time exerted no significant effect upon the PV. This shows that during the settling time, there is no chemical or biochemical reaction that can contribute to the breakdown of triglycerides into free fatty acid. Moreover, during this phase, the temperature that favours triglyceride hydrolysis is gradually going down, and the time is not long enough to favour microorganism development which could secrete lipases to accelerate triglyceride breakdown [25]. Indeed, only the quadratic effect of viscera/water ratio was significant on yield and AV, while the quadratic effect of cooking time was significant on PV. This may be due to heating in the aqueous medium which favours fixation of oxygen molecules on the triglyceride structure. Table 5 also shows that the proposed quadratic models for AV, PV and yield explain the variability of the data to a large extent, with coefficients of determination,  $R^2$  around or higher than 0.90. However, for PV, lower  $R^2$  was obtained, 0.85.

**Table 4.** Analysis of variance(ANOVA)for Response Surface Quadratic Model on yield extraction by cooking treatment

<b>ANOVA for response surface quadratic model by cooking treatment</b>									
	<b>On yield extraction</b>			<b>On acid value</b>			<b>On peroxide value</b>		
<b>Source</b>	<b>Sum of sq.</b>	<b>F- Value</b>	<b>P- Value</b>	<b>Sum of sq</b>	<b>F- Value</b>	<b>P- Value</b>	<b>Sum of sq</b>	<b>F- Value</b>	<b>P- Value</b>
<b>X<sub>0</sub></b>	953.12	9.55	<b>0.0035</b>	1100.92	9.2	<b>0.004</b>	6.34	4.71	<b>0.0267</b>
<b>X<sub>1</sub>- Cooking time</b>	122.64	11.06	<b>0.0127</b>	164.2	12.35	<b>0.0098</b>	0.2359	1.58	0.2494
<b>X<sub>2</sub>- Viscera/water ratio</b>	569.95	51.4	<b>0.0002</b>	473.12	35.58	<b>0.0006</b>	2.66	17.79	<b>0.0039</b>
<b>X<sub>3</sub>- Settling time</b>	86.34	7.79	<b>0.0269</b>	0.084	0.0063	0.9389	0.1681	1.12	0.3243
<b>X<sub>1</sub>X<sub>2</sub></b>	20.8	1.88	0.2131	241.67	18.17	<b>0.0037</b>	0.605	4.05	0.0842
<b>X<sub>1</sub>X<sub>3</sub></b>	13.26	1.2	0.3103	0.1225	0.0092	0.9262	0.245	1.64	0.2414
<b>X<sub>2</sub>X<sub>3</sub></b>	1.71	0.1543	0.7061	0.1225	0.0092	0.9262	0.245	1.64	0.2414
<b>X<sub>1</sub><sup>2</sup></b>	6.83	0.6156	0.4584	0.5838	0.0439	0.84	1.45	9.68	0.017
<b>X<sub>2</sub><sup>2</sup></b>	111.08	10.02	0.0158	220.93	16.61	0.0047	0.7222	4.83	0.064
<b>X<sub>3</sub><sup>2</sup></b>	20.51	1.85	0.216	0.088	0.0066	0.9374	0.0048	0.0322	0.8626
<b>Residual</b>	77.61			93.09			1.05		
<b>Lack of Fit</b>	63.01	1.73	0.4062	93.09			1.05		
<b>Pure Error</b>	14.61			0			0		
<b>Cor Total</b>	1030.74			1194.01			7.38		
<b>R<sup>2</sup>(%)</b>	92.47			92.20			85.82		
<b>Adjusted R<sup>2</sup>(%)</b>	82.79			82.18			67.58		
<b>Predicted R<sup>2</sup></b>	0.446			0.384			-0.329		
<b>Adeq Precision</b>	10.35			9.35			7.06		
<b>AAD</b>	-			-			0.05		
<b>BF</b>	-			-			1.04		

X<sub>0</sub> represents the model's constant term; X<sub>1</sub>, X<sub>2</sub>, and X<sub>3</sub> the linear effects (for cooking time, viscera mass/water volume ratio and settling time respectively); X<sub>1</sub>X<sub>2</sub>, X<sub>1</sub>X<sub>3</sub> and X<sub>2</sub>X<sub>3</sub> are the different interactions and X<sub>1</sub><sup>2</sup>, X<sub>2</sub><sup>2</sup> and X<sub>3</sub><sup>2</sup> the quadratic effects.

The Predicted R<sup>2</sup> of 0.446, 0.384 and -0.329 for extraction yield, acid value respectively are not as close to the Adjusted R<sup>2</sup> as one might normally expect; i.e., the difference is more

than 0.2. This may indicate a large block effect or a possible problem with the model and/or data. A negative Predicted  $R^2$  form peroxide value implies that the overall mean may be a better predictor of response than the current model. In some cases, a higher order model may also predict better. Things to consider are model reduction, response transformation, outliers.

From these results, it is indicated that the different processing conditions (cooking time, viscera/water ratio and settling time) were required for the optimisation of yield, AV and PV. It is observed that high cooking time enhances the oil yield but reduces its quality in terms of acidity and hydroperoxides concentration [26] thus the need of doing an optimization.

### 3.2.1 Optimization procedures

Multiple response optimizations were used to assess the optimum levels of the parameters which could achieve the desirable response areas [27]. Different colored oils were obtained (picture 1) and besides the numerical optimization, the 3D plots which were advocated for the graphical interpretation of the interaction effect of independent variables on the dependent variables [28] were also considered using the Design Expert software to locate the exact optimum point of the independent variables and to obtain the overall joint optimized values. The overall optimal values were found as: temperature 95°C, cooking time 17.02min, viscera mass/water ratio 100:152 g/ml and settling time 96min.

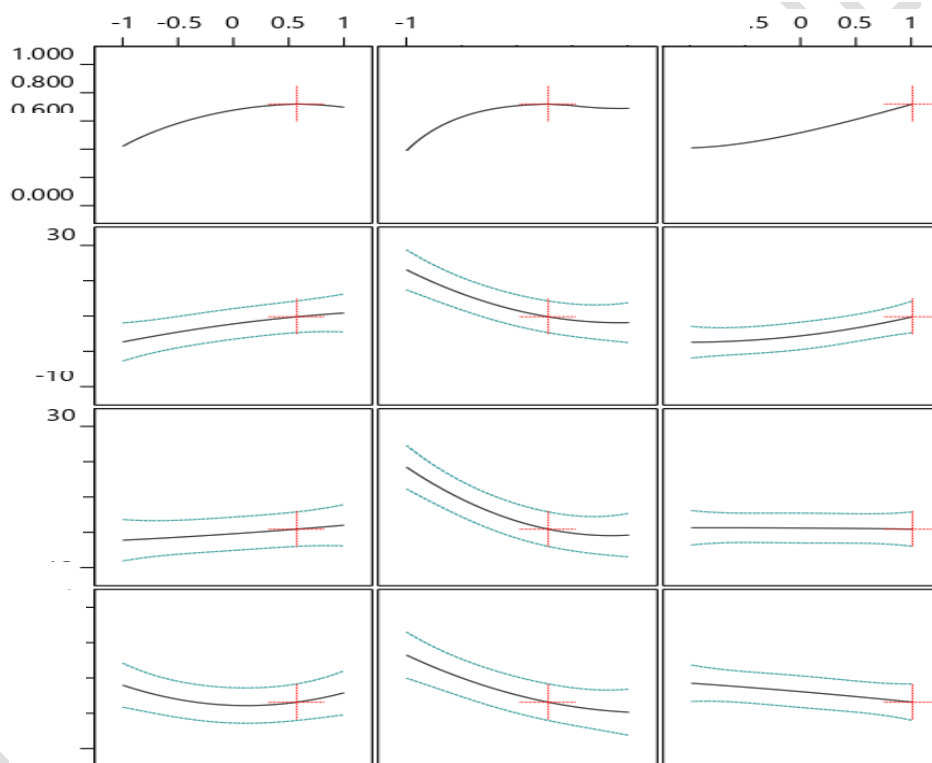


**Picture 1.** Viscera oil extracted at viscera/water ratio of 100:100, at cooking time 25min (a), cooking time 15min (b) and cooking time 10min (c).

The differences of color in bottle a) b) and c) could be due to the cooking time. Dark colored oil (a) corresponds to the cooking time of 25min, the less dark one (b) corresponds to the

cooking time of 17min, and the light-colored oil (c) corresponds to the cooking time of 10min. The dark color of oils a) and b) may be due to the caramelization of the proteins present in the viscera during cooking [29]. However, the more the viscera are exposed to heat, the more by diffusion the walls of the viscera cells are weakened and therefore facilitates the release of lipid molecules from the capillary walls, hence the increase in the quantity of oil when the cooking times is longer.

The contour plot of each factor showing their influence in responses was plotted and presented in figure 2.



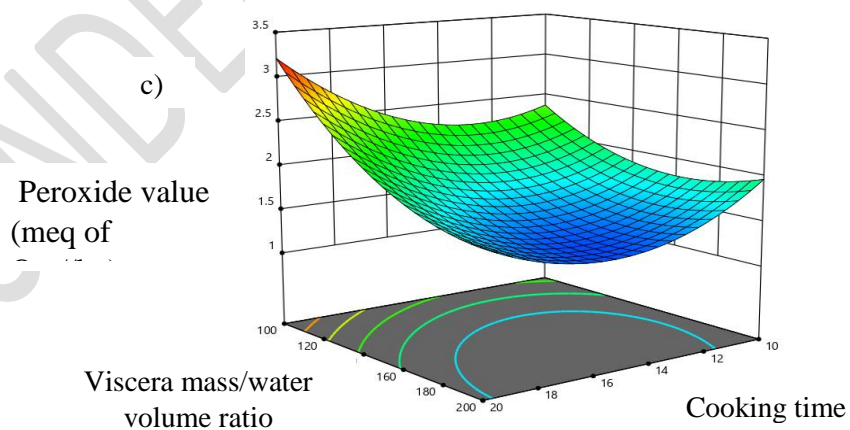
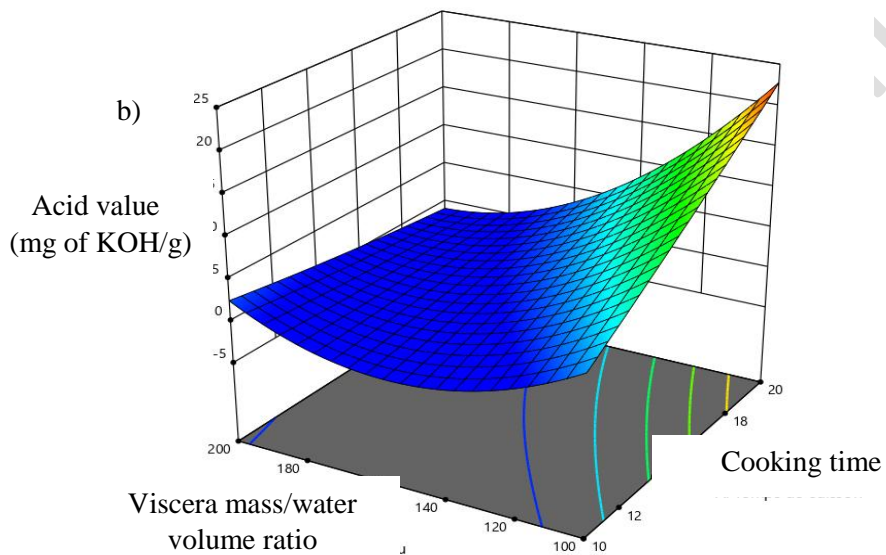
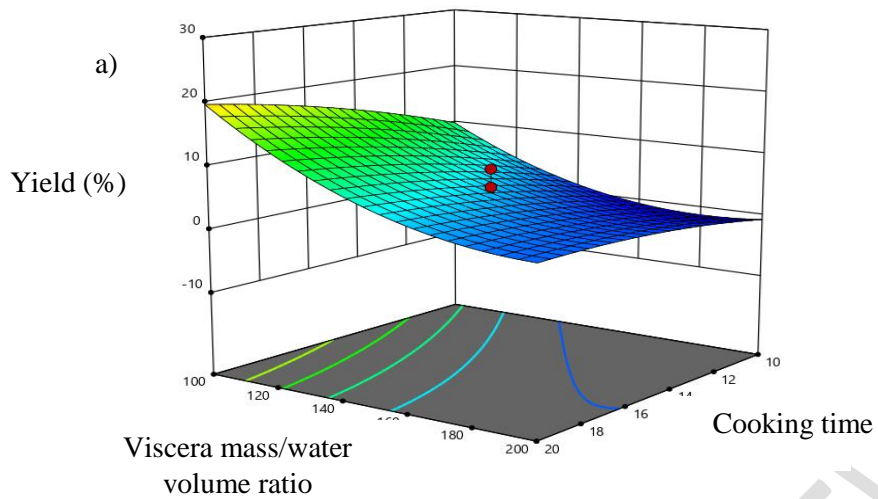
**Fig. 2.** Factors plot of optimum conditions of the extraction yield (%), acid value (mg of KOH/g) and peroxide value (meq of O<sub>2</sub>//kg).

This figure illustrates the effect of the parameters studied on the yield and quality of fish oil. It can be observed that the Viscera mass/water reduces the acid value which is a positive effect, while the cooking and settling time have no or very slight effects. It is to be noted that the more the acid value is low, the more the fish oil quality is good [30, 4]. The same trend is

observed with the fish oil yield. Also, the result discussed above for peroxide value is also illustrated by the trends of the curves.

These results were also represented by the response surface plots (Figure 3) to have a better view of each of the parameters on various responses in three dimensions. The plots present the simultaneous effect of the cooking time and the viscera/water ratio on the evolution of the extraction yield, the acid value, and the peroxide value.

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**Fig. 3.** Surface plot of factors influencing on sardine viscera oil (a) extraction yield, (b) acid value and (c) peroxide value.

### **3.2.2 Effect of cooking time**

The effect of cooking time on oil extraction yield, acid value and peroxide value from sardine viscera was investigated and the results are showed in Figure 3. It was observed that the increasing of cooking time up to 20 min, increase the percentage of extracted oil as well as the peroxide value. However, increasing cooking time up to 20 min reduced the acid value. This finding could be explained by the fact, growing the cooking time breaks the membrane of the adipocyte cells, which therefore liberated their content in the environment and increased the oil yield. Similar result was reported by Oliveira et al. [31] and El-Rahman et al. [32], who found that boiling up to 50 minutes was good for extracting the oil from the tilapia viscera.

### **3.2.3 Effect of ratio viscera mass/water**

The effect of viscera mass/water ratio from 100:100 to 100:200 g/ml on oil extraction yield, acid value and peroxide value from sardine viscera was investigated. It was observed that increasing the ratio and therefore the volume of water in the mixture decrease the extraction yield (from 34.8 to 0.1%), the acid value (from 23.66 to 0.95 mg of KOH/g) and the peroxide value (from 3.2 to 1,2 meq of O<sub>2</sub>//kg). It may be due to the low polarity between the water molecules and the lipid. The results obtained in the current study is in line with the report of El-Rahman et al. [32], who pointed out that water was not an appropriate solvent for the extraction of Mackerel and Tilapia oils because it leads to a low yield of 3% and 10% respectively.

### **3.2.4 Effect of settling time**

The effect of settling time on extraction yield, acid and peroxide value was investigated. It appears that increasing the settling time contributes to increasing the extraction yield (from 0.1-34.8%) and reduces the peroxide index (from 3.2-1.2 meq/kg). However, increasing the settling time has no effect on the acid value. These results can be explained by the fact that during decantation, the lipid molecules continue to diffuse out of the capillary membranes of the viscera.

### 3.2.5 Optimal condition

In order to determine the appropriate time that would enable the obtention of high quantity of oil and good quality, the optimization step was conducted. The optimum values were obtained by solving the regression equation analytically. The optimal cooking treatments were predicted as follows: temperature at 95°C, cooking time 17.02min, viscera mass/water volume ratio 100:152 g/ml and settling time 96min. At these predicted optimal conditions, the extraction yield was 25.15%, the acid value 2.5 mg KOH/g and the peroxide value 1.9 meq/kg. The experimental test of these conditions provided an optimum oil yield of 25.10%, an acid value of 1.35 mg KOH/g and a peroxide value of 1.20 meq/kg. This result indicates an adequate signal. The yield extraction in the optimal condition is higher than 10.90% obtained by the local fish oil producers in the Far North of Cameroon [4], and 12% obtain by García-Moreno et al. [26] who also extracted oil fish from sardine (*S. pilchardus*) by hydraulic pressing method in Spain. The differences in the yield value of the oil could reflect the state of viscera sample and its condition towards physical extraction (temperature, ratio, and cooking time) profile during extraction [33].

### 3.2.6 Degree of Oxidation of the extracted oil

The water content of oil determines the quality of oil and its stability during storage. In table 5, it can be seen that the water content of the oils after optimization is comparable to that of the oils purchased in the markets of the Far North of Cameroon, which was 0.38 %. The values found are higher than those reported by El-Rahmanet al. [32] (0.14%) on Tilapia viscera. Codex standards [34], recommend that fish oil should be free from water (0 %). The presence of water can be justified by the extraction process (cooking).

The acid value gives an indication of the oxidative state and quality of an oil Abdulkadir et al. [30]. It is used as a guide in quality control as well as in monitoring oil degradation during storage. The acid value obtained in this study was 1.35mg KOH/g, lower than 6.93 mg KOH/g determined in the oil produced traditionally in the same localities (table 5). The

improved process put in place permitted to reduce the oxidation of the oil and bring down the acid value to the accepted standard range of Codex Alimentarius [34] which is a set of standards, guidelines and codes of practice adopted by the food and agriculture Organization of the United Nations (FAO) for fish oils.

**Table 5.** Extraction yields and physicochemical parameters of viscera oils.

Parameters	Local	Optimal values	Codex [34] and ASTM standards [35]
Extraction efficiency	10 ± 2 <sup>b</sup>	25 ± 2 <sup>a</sup>	
Acid value (mg KOH/g oil)	6.93 ± 0.50 <sup>a</sup>	1.35 ± 0.01 <sup>b</sup>	≤3
Peroxide value (meq/kg oil)	8.47 ± 0.01 <sup>a</sup>	1.20 ± 0.01 <sup>b</sup>	≤5
Iodine value (g/100 g oil)	83 ± 3 <sup>a</sup>	87 ± 2 <sup>a</sup>	160-200
Saponification value (mg/g oil)	255 ± 3 <sup>a</sup>	185 ± 2 <sup>b</sup>	180-195
Refractive index	1.46 ± 0.01 <sup>a</sup>	1.47 ± 0.01 <sup>a</sup>	1.47-1.48
Water content	0.38 ± 0.01 <sup>a</sup>	0.35 ± 0.01 <sup>b</sup>	0
Relative density	0.919 ± 0.002 <sup>a</sup>	0.911 ± 0.002 <sup>b</sup>	0.920-0.930

*Value on the same line with the same superscript letter are not significantly different at P= 0.05; n = 3. Values are expressed as means ± standard deviation*

PV indicate the initial rancidity of the oil [36]. The smaller the peroxide number the better is the quality of the oil. PV can be used to monitor the fats and oils quality and stability [33]. The peroxide value of 1.20 meq/kg of oil obtained during this study is in line with that of Salih et al. [14], who obtained out a peroxide value of 1.8 meq/kg for the Common Carp. This value is also lower than that found by Mkademand Kaanane [37](4.61 mEq active Oxygen/kg oil) on sardine viscera oils extracted by hydraulic pressing at 85°C. The maximum value of the peroxide value of authorized fish oils is less than 10 meqO<sub>2</sub>/kg oil [34]. Consequently, the oil obtained in this study can be safe for consumption because it is less than 10.

The iodine value measures the degree of unsaturation of oil, it determines the stability of oils to oxidation, and makes it possible to qualitatively determine the overall unsaturation of the fat [38]. The value of 87.68 g/100 g of oil obtained during this study is close with that of Salih et al. [14], who pointed out an iodine value of  $85.16 \pm 2.011$  g/100 g for the Common Carp. This result may be due to the fact that the oils from the optimized process would be richer in long-chain polyunsaturated fatty acids such as eicosapentaenoic acid, docosahexaenoic acid [37].

The high number of saponification value indicates that the oil contains fatty acid and triacylglycerol of low molecular weight value [39]. Fish oil having low saponification value is less prone to rancidity. The values recorded in the current study at the optimal point (185.45 mg/g oil) are compared than the value of 187.45 mg/g oil obtained by Salih et al. [14] with viscera carp. The values obtained for the oils sold in the Far North (255.12 mg/g oil) do not comply with the Codex standards [34]. This can be justified by the lack of control of unit operations such as heating which acts on the length of the fatty acid chains.

The refractive index is used to determine the purity of oils where values increase with the percentage of unsaturated fatty acids in the oil Bako et al. (2017). The refraction index recorded in the current study at the optimal processing conditions was 1.47 and the value of obtained by women producers was 1.46 in the far North Region. The values of the refractive index were closed for both extracted methods.

#### **4. CONCLUSION**

The aim of this study was to improve the traditional sardine fish oil extraction in the far North region of Cameroon by determining the optimum processing conditions of viscera oil extracted from sardine (*S. pilchardus*). The optimal values of variables were found to be 95°C, 17.02min and 100:152 g/ml respectively for temperature, cooking time and viscera mass/water. At this optimized condition, the various responses recorded were: extraction yield of 25.10%, acid value of 1.35 mg KOH/g, and the peroxide value of 1.20 meq O<sub>2</sub>/kg.

It can be concluded that the optimization of the traditional process improves the extraction yield by 2.3 times and best preserves the qualities of the sardine viscera oil. The relationship between the actual and predicted data indicates that the response model was adequate to reflect the expected optimization. This will therefore help fish oil producers in the Far North region of Cameroon.

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