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# LOREXP

LOCAL RESOURCE EXPLOITATION

### Physicochemical characteristics and antibacterial activity of cooked and dried *Lutjanus dentatus* (Dumeril, 1860) oils

### *Caractéristiques physico-chimiques et activité antibactérienne des huiles de Lutjanus dentatus cuites et séchées (Dumeril, 1860)*

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#### ABSTRACT:

The limits of antibiotic prompted researchers to explore foods components as antimicrobial. The aim of the present study was to determine the physicochemical characteristics and antibacterial activity of oils extracted from the fat tissues of *Lutjanus dentatus* after cooked and dried against food poisoning bacteria. The oils were extracted from the adipose tissue by drying at 45 °C for 24 hours and by cooking in a pressure cooker at 95 °C for 20 min followed by pressing. Subsequently, the extraction yield and the physicochemical characteristics of the oils were analyzed through the quality indices and FTIR spectra determination. The antibacterial activity of the oils, their emulsion as well as their interactions with some common antibiotics were evaluated by the broth microdilution method. *L. dentatus* oil adipose tissue extraction yield obtained by cooking at 95 °C was high (66.83 ± 3.1 %) compared to that obtained after drying at 45 °C (55.5 ± 2 %). The oil extracted from *L. dentatus* adipose tissue by drying at 45 °C showed peroxide (9.76 ± 1.19 meqd'O<sub>2</sub>) and anisidine indices (40.94 ± 0.8) higher than those obtained by cooking at 95 °C (6.56 ± 0.40 meq d'O<sub>2</sub> and 37.85 ± 0.34 respectively). However, the acid, iodine and thiobarbituric acid values of oils extracted using the two methods were not significantly different  $P \leq 0.05$ . The FTIR profile provided information on the functional groups present in the oil and enable to appreciate the variation of the peaks compared to the quality indices. The antibacterial test showed that all the oils studied all had antibacterial activity. The best spectrum of action against 23 bacteria tested (16 ≤ MIC ≤ 256 mg/mL) was noted with the oil extracted by cooking at 95 °C. Regardless of the extraction method, emulsions have better antibacterial activity compared to the oils (0.39 ≤ MIC ≤ 12.5 mg/mL). Moreover *L. dentatus* oil adipose tissue potentiated the activity of Ciprofloxacin, Tetracyclin, Gentamicin, Amoxicilin and Chloramphenicol on the bacterial strains tested. These results showed the effectiveness of the *L. dentatus* oils extracted against the bacteria strains tested and demonstrated their eventual antimicrobial properties.

**Keywords:** *Lutjanus dentatus*, Quality index, Antibacterial activity, Cooking, Drying.

#### RÉSUMÉ :

Les limites des antibiotiques ont incité les chercheurs à explorer les composants alimentaires comme antimicrobiens. Le but de la présente étude était de déterminer les caractéristiques physico-chimiques et l'activité antibactérienne des huiles extraites des tissus adipeux de *Lutjanus dentatus* après cuisson et séchage contre les bactéries d'intoxication alimentaire. Les huiles ont été extraites du tissu adipeux par séchage à 45 °C pendant 24 heures et par cuisson dans un autocuiseur à 95 °C pendant 20 min suivi d'un pressage. Par la suite, le rendement d'extraction et les caractéristiques physico-chimiques des huiles ont été analysés à travers les indices de qualité et la détermination des spectres FTIR. L'activité antibactérienne des huiles, leur émulsion ainsi que leurs interactions avec certains antibiotiques courants ont été évaluées par la méthode de microdilution en bouillon. Le rendement d'extraction du tissu adipeux de l'huile de *L. dentatus* obtenu par cuisson à 95 °C était élevé (66,83 ± 3,1 %) par rapport à celui obtenu après séchage à 45 °C (55,5 ± 2 %). L'huile extraite du tissu adipeux de *L. dentatus* par séchage à 45 °C a montré des indices de peroxyde (9,76 ± 1,19 meqd'O<sub>2</sub>) et d'anisidine (40,94 ± 0,8) supérieurs à ceux obtenus par cuisson à 95 °C (6,56 ± 0,40 meq d'O<sub>2</sub> et 37,85 ± 0,34 respectivement). Cependant, les valeurs d'acide, d'iode et d'acide thiobarbiturique des huiles extraites à l'aide des deux méthodes n'étaient pas significativement différentes de  $P \leq 0,05$ . Le profil FTIR a fourni des informations sur les groupes fonctionnels présents dans l'huile et permet d'apprécier la variation des pics par rapport aux indices de qualité. Le test antibactérien a montré que toutes les huiles étudiées avaient toutes une activité antibactérienne. Le meilleur spectre d'action contre 23 bactéries testées (16 ≤ MIC ≤ 256 mg/mL) a été noté avec l'huile extraite par cuisson à 95 °C. Quelle que soit la méthode d'extraction, les émulsions ont une meilleure activité antibactérienne par rapport aux huiles (0,39 ≤ MIC ≤ 12,5 mg/mL). De plus, le tissu adipeux de l'huile de *L. dentatus* a potentialisé l'activité de la ciprofloxacine, de la tétracycline, de la gentamicine, de l'amoxiciline et du chloramphénicol sur les souches bactériennes testées. Ces résultats ont montré l'efficacité des huiles de *L. dentatus* extraites contre les souches bactériennes testées et ont démontré leurs éventuelles propriétés antimicrobiennes.

**Mots clés :** *Lutjanus dentatus*, Indice de qualité, Activité antibactérienne, Cuisson, Séchage.

## 1. INTRODUCTION

Food provides the body with energy and nutrients it needs to function. However, they are capable of carrying infectious microorganisms which are the cause of food borne illnesses very common in our environment (Ruasse, 2018; OMS, 2010). The most effective drugs used against these bacterial infections are antibiotics. However, the abusive and inappropriate use of these has led to resistance to many antibiotics once reputed (Desobry et al., 2016).

In the search for alternatives, some researchers have opted for the use of essential oils, plant extracts (Belletti et al., 2004) and fish oils (Chitra and Radhakrishnan, 2011; Mouokeu et al., 2018). As such, fish oils have so far revealed several pharmacological and more precisely antibacterial properties (Molla et al., 2015).

Annual world production of fish from fisheries and aquaculture is estimated at 171 million tones (FAO, 2018). A large part is processed and then used for human consumption (FAO, 2018). These processing steps generate a significant amount of offal and other by-products made up of viscera, bones, fins, skins and fat. Those co-products are still an under-exploited source of nutrients with useful therapeutic and functional properties for humans (FAO/DANIDA, 2001). At the pre-treatment unit of the Youpwe landing stage in the city of Douala, Cameroon, the adipose tissue of *Lutjanus dentatus* are generated in large quantities and directly discharged into the environment, causing pollution problems. This work was therefore initiated in order to explore the properties of oils extracted from the fats of this fish.

## 2. MATERIALS AND METHODS

### 2.1. Materials

#### 2.1.1. Fish

The body of *Lutjanus dentatus* (Snapper carp) locally referred to as "Wanga" is high, the height averaging 32.60 % of standard length. The pelvic fins are short and clear, not reaching the anus. The second anal spine is shorter than the third and is not stronger. The scales in transverse row, counted between the 5<sup>th</sup> dorsal spine and the lateral line, are larger, being 7 or more rarely 8. It has an average of 8 gill rakers on the lower part of the first brancial arch.

#### 2.1.2. Adipose tissue collection

The adipose tissue used for oil extraction was removed from the abdominal part by evisceration of *Lutjanus dentatus* collected from fish cleaners at the Youpwe landing stage in Douala. They were stored under ice in a cooler and transported directly to the Laboratory. Fish identification was carried out by ichthyologists from the Fisheries Resources Laboratory of the Institute of Fisheries and Aquatic Sciences at the University of Douala.

#### 2.1.3. Microorganisms

The bacteria used in this study consisted of Gram + and Gram- bacteria, including strains of *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Salmonella enterica serovar typhi*, *Citrobacter freundii* and *Yersinia enterocolitica*. The reference strains came from the Laboratory of Microbiology and Antimicrobial Substances of the University of Dschang, while the clinical strains came from the Laboratory of Biochemistry of Natural Substances of the Department of Biochemistry of the University of Douala.

## **2.2. Methods**

### **2.2.1. Oils extraction**

#### *Drying*

636 g of the fish adipose tissue was cut into pieces and stored in an thermostat oven model DHG-9101-2SA at temperature of 45 °C for 24 hours during which oil was collected.

#### *Cooking*

The extraction by cooking at 95 °C was carried out in a household autoclave into which 300 g of the fish adipose tissue was introduced, brought to a temperature of 95 °C for 20 min. The oil that emerged during cooking from the tissue was then collected.

In both cases, the oils obtained were weighed and stored in opaque containers at 4 °C in the refrigerator.

### **2.2.2. Determination of fish oil quality**

The acid, iodine and anisidine indices were evaluated according to standard methods recommended by the French Association for Standardization (AFNOR, 1981). The peroxide index was determined according to the spectrophotometric method recommended by the IDF (1991). The Thiobarbituric Acid Index was determined using the method recommended by the American Oil Chemist Society (AOCS, 1987).

### **2.2.3. Fourier Transform Infrared (FTIR) Spectra Analysis**

IR spectra between 3800 and 500  $\text{cm}^{-1}$  were recorded using a tensor 27 (Bruker, Wissembourg, France) equipped with an ATR prism crystal accessory and MCT detector (Mercury Cadmium Telluride). The spectra resolution was 4  $\text{cm}^{-1}$ . Measurements were performed at RT using approximately 2  $\mu\text{L}$  of the fish oils, which were placed on the surface of the ATR crystal and pressed with a flat-tip plunger until spectra with suitable peaks were obtained. The background was subtracted using the spectrum software OPUS version 6.3.2 (Perkin-Elmer Inc.).

### **2.2.4. Antibacterial test**

#### *Antibacterial activity evaluation of fish oils and emulsion*

The antibacterial activity of the oils was evaluated with respect to bacterial strains by the broth microdilution method (CLSI, 2015). For each bacterial strain, the inocula was prepared from overnight bacterial culture and adjusted to  $1.5 \times 10^6$  CFU/mL in broth Mueller Hinton using Mc Farland Scale (Oussou et al., 2008). The oil stock solutions were prepared at 1024 mg/mL in a 5 % Tween 80. Also, the stock solution of Ciprofloxacin was prepared at 256  $\mu\text{g/mL}$ . Moreover, oil emulsions were prepared as previously described by Prinderre et al. (1998). For this purpose, 0.5 g of oil was stirred in a beaker containing 0.5 g of tween 80 using a magnetic stirrer at 800 rpm for 30 min. Subsequently, 9 mL of sterile distilled water adjusted to a flow rate of 2 mL/min through a burette were added to the reaction medium maintained under a magnetic stirrer for 2 hours. The stock émulsion obtained at a concentration of 50 mg/mL was packed in an opaque bottle and left to stand for 2 days at 4 °C temperature before use. As far as the antibacterial test is concerned, the Minimum Inhibitory Concentrations (MICs) and the Minimum Bactericidal Concentrations (MBCs) made it possible to assess the antibacterial activity. The tests were performed in 96 microtitre plates. The final volume in each well was 200  $\mu\text{L}$ . Test sample concentrations were between 2 and 256 mg/mL. Ciprofloxacin was used as a positive control at concentrations ranging between 2 and 256  $\mu\text{g/mL}$ . Tests were carried out in triplicate and repeated thrice. The MICs was defined

as the lowest test sample that prevents development of the yellow dye of *P*-iodonitrotetrazolium chloride (INT; 0.2 mg/mL) to a pink color (Mouokeu et al., 2014). The MBC values were determined by adding 50 µl aliquots of the preparations which did not show any growth after incubation during MIC assays to 150 µl broth culture medium. These preparations were incubated at 37 °C for 24h. The MBCs values were defined as the lowest concentration of test sample that prevent color change of INT as mentioned above. Following MBCs determinations, the CMB/CMI allowed to reveal either the bactericidal effect (CMB / CMI ≤ 4) or the bacteriostatic effect (CMB / CMI > 4) (Djeussi et al., 2013).

### 2.2.5. Fish oils –antibiotics interaction study

100 µl of Mueller Hinton broth culture medium was introduced into each well of a 96-well microtitre plate, followed by the addition of 100 µl of antibiotic. A subsequent dilution was thereafter made to obtain final antibiotic concentrations less than or equal to the MIC. 100 µL of oil solution was introduced into each well. The stock solutions of oil were prepared so as to obtain final concentrations in the wells equal to MIC and MIC × 2. The content of each well was diluted by adding 100 µL of inoculum. The MIC was determined as mentioned above (Eloff, 1998). The interaction between each oil sample and the antibiotics including Chloramphenicol, Amoxicillin, Tetracyclin, and Gentamycin was determined by calculating the Fractional Inhibitory Concentrations (CIF) using equation 1:

$$CIF = CMI_{ATB + Oil} / CMI_{ATB \text{ alone}} \tag{1}$$

Four types of interactions were recorded according to the value of the Fractional Inhibitory Concentration: synergistic interaction (CIF ≤ 0.5); additive interaction (0.5 < CIF ≤ 1); indifferent interaction (1 < CIF ≤ 4) and antagonistic interaction (CIF > 4) (Coutinho et al., 2010).

### 2.3. Statistical Analysis

The results of the oil quality indices were expressed with more or less standard deviation means. The means was compared by the ANOVA I test to the 5 % probability threshold using GraphPad InStat version 2000 software.

## 3. RESULTS AND DISCUSSION

### 3.1. Influence of oil extraction method on yield

The extracting method oil from the adipose tissue of *L. dentatus* greatly influences the extraction yield in the samples (Table 1). The best yield (66.83 %) was achieved by cooking.

**Table 1:** Extraction yield of the oil from the adipose tissue of *Lutjanus dentatus* according to the extraction method.

Extraction methods	Sample Mass (g)	Mass of oils extracted (g)	Volume of oils extracted (mL)	Extraction yield % (w/w)
Drying at 45 °C	636	353	405	55.50
cooking at 95 °C	300	200.5	230	66.83

### 3.2. Influence of extraction method on oils indices

Oil quality indices varied depending on the extraction method used (Table 2). The oil obtained by drying at 45 °C showed higher peroxide and anisidine indices than those exuded at 95 °C. However, the acid, iodine and thiobarbituric acid indices of oils extracted by both methods were comparable.

**Table 2:** Quality index of the oil from adipose tissue of *Lutjanus dentatus* according to the extraction method

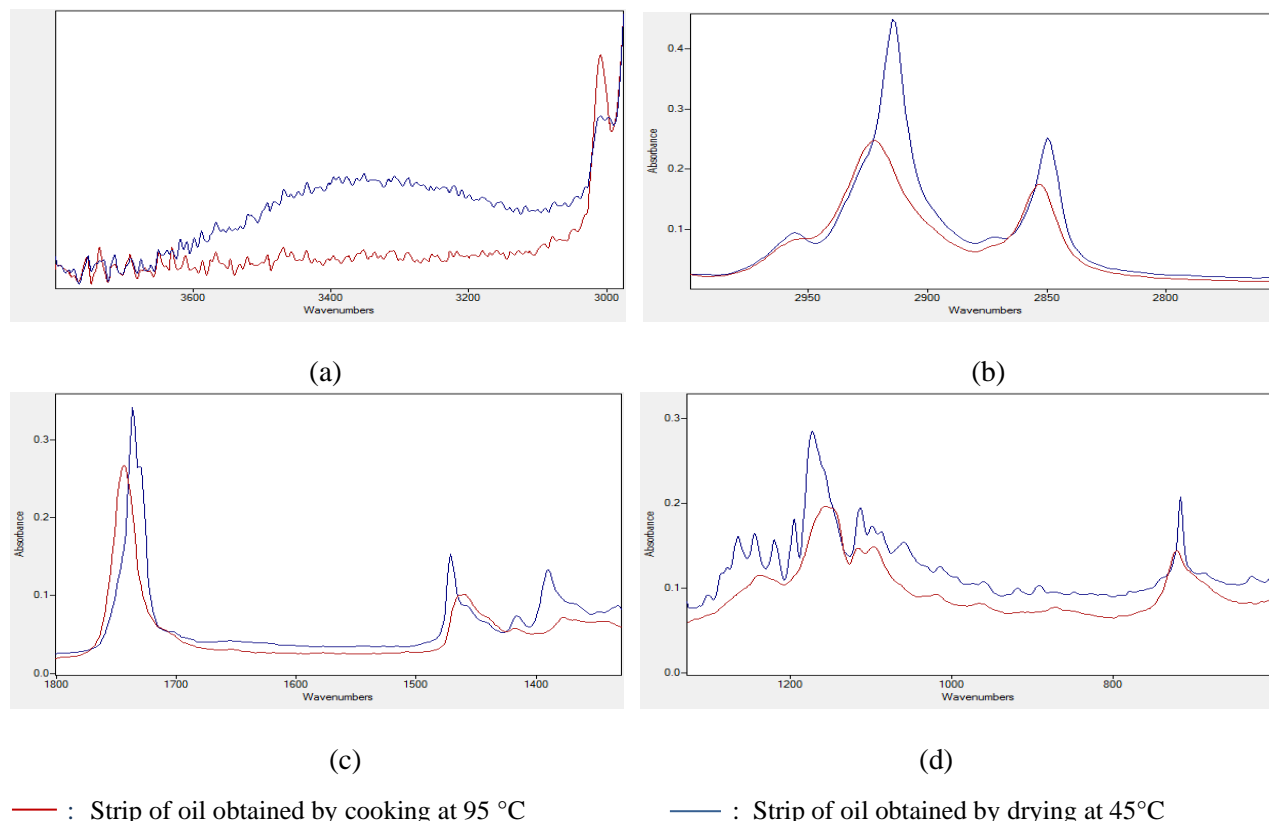
Extraction methods	Acide Index (mg KOH/g)	Iodine index (g I2/100 g)	Peroxyde index (meq d'O2/kg)	Thiobarbituric acide index (µmol MDA/kg)	Anisidine index
Drying at 45 °C	3.73±1.53 <sup>a</sup>	91.55±7.10 <sup>a</sup>	9.76±1.19 <sup>b</sup>	1.99±0.28 <sup>a</sup>	40.94±0.87 <sup>b</sup>
cooking at 95 °C	3.24±0.54 <sup>a</sup>	102.47±5.08 <sup>a</sup>	6.56±0.40 <sup>a</sup>	2.21±0.10 <sup>a</sup>	37.85±0.34 <sup>a</sup>

In the same column, the numbers bearing the same letters are not significantly different (P> 0.05), (n = 3)

### 3.3. Analytical changes occurring in the oil of the adipose tissue of *L. dentatus* during extraction by drying at 45 °C and cooking by infrared spectroscopy (FTIR)

Infrared spectroscopy was used to identify the functional groups present in the organic components of samples of *Lutjanus dentatus* oils. From a global point of view, these spectra have the same appearance; since coming from the same fish.

The spectra obtained between 3800 and 500 cm<sup>-1</sup> on oils extracted by drying at 45 °C and cooking, are presented in Figure 1.



**Figure 1:** Selected regions (a)-(d) of FTIR spectra of lipids extracted from *L. dentatus* as function of extraction methods

The weak band associated with the hydroxyl groups formed during the oxidation appears in Figure 1a close to  $3350\text{ cm}^{-1}$ . It is observed that the peak of the oil obtained by drying at  $45\text{ °C}$  is greater than that obtained by cooking. This means that the quantity of hydroperoxide formed in oil obtained by drying at  $45\text{ °C}$  is more important than that content in oil obtained after cooking. Consequently, oil obtained by drying is more oxidized than that obtained after cooking.

The peak at  $3008\text{ cm}^{-1}$ , linked to the  $\text{C sp}^2\text{-H}$  stretching vibration of the cis double bond ( $=\text{CH}$ ), provides information on the degree of lipid unsaturation. This peak is greater in the oil obtained after cooking (Figure 1a). This implies that oil obtained after cooking contains more insaturation than the oil obtained after drying, thus confirming the previous results.

The two peaks just below 3000 ( $2955\text{-}2922\text{ cm}^{-1}$  and  $2853\text{-}2849\text{ cm}^{-1}$ ) can both be attributed to the absorption caused by the asymmetrical and symmetrical stretching vibrations of the methyl and methylene groups (Figure 1b). For these two peaks, the absorbances were greater in the oil obtained by drying at  $45\text{ °C}$ . A high absorbance indicates a significant appreciable content of the concentration of the functional groups  $\text{CH}_3$  and  $\text{CH}_2$ . During the primary oxidation,  $-\text{CH}=\text{CH}-$  react to form  $-\text{CH}_2-\text{CH}(\text{OOH})-$  and during the secondary oxidation  $-\text{CH}_2-\text{CH}(\text{OOH})-$  can react to form  $-\text{CH}_3$  et  $-\text{CHO}$ ; This justify respective increase of group numbers of methylene et methyl.

The stretch vibration band attributable to the  $\text{C}=\text{O}$  group of triglycerides (aldehyd, ketone, ester) was found at around  $1742\text{ cm}^{-1}$  (Figure 1c). The high peak in *Lutjanus dentatus* oil obtained by drying at  $45\text{ °C}$  compared to the other could mean that this sample contains higher carbonyl groups, probably aldehydes and ketones, which are secondary oxidation products derived from hydroperoxides. This may justify the higher anisidine index in *Lutjanus dentatus* oil obtained by drying at  $45\text{ °C}$  (Table 2).

The bands appearing at  $918\text{ cm}^{-1}$  (Figure 1d) are linked to the vibration in the molecules analyzed of the double trans bonds, while those appearing at  $716\text{ cm}^{-1}$  (figure 1d) are linked to the vibration in the molecules analyzed of the double cis bonds.

### 3.4. Influence oil extraction method on antibacterial properties

The antibacterial activity of the oils varied depending on the extraction method, bacterial strains and the preparation technique of the test sample (Table 3). Both oils showed comparable antibacterial activity on *Shigella flexneri*  $64 \leq \text{MIC} \leq 256\text{ mg/mL}$ , *Citrobacter freundii*  $16 \leq \text{MIC} \leq 256\text{ mg/mL}$ , *Yersinia enterocolitica*  $32 \leq \text{MIC} \leq 128\text{ mg/mL}$ , *Staphylococcus aureus* and in particular on methycilin resistant strains  $\text{MIC}=128\text{ mg/mL}$ . With MICs values of  $32 \leq \text{MIC} \leq 128\text{ mg/mL}$  on *E. coli* strains and confirmed activity on all strains of *Salmonella enterica serovar typhi*, the oil obtained after cooking was more active compared to that obtained by drying at  $45\text{ °C}$  (MIC). In addition, the oil obtained by drying at  $45\text{ °C}$  was more active on strains of *Klebsiella pneumoniae* with  $16 \leq \text{MIC} \leq 64\text{ mg/mL}$ .

In general, the MICs value of the emulsions was lower regardless of the oil extraction method ( $0.39 \leq \text{MICs} \leq 12.5$ ), reflecting an increase antibacterial activity. The activity of these emulsions increases by 20 times on most bacteria with the oil obtained by drying at  $45\text{ °C}$ .

Regardless of the bacteria strain, the temperature of oil preparation and the technique of preparation of the stock solution to be tested, the MBC/MIC ratio was greater than or equal to 4 in general, reflecting a bacteriostatic activity of adipose tissue oil from *L. dentatus*.

**Table 3:** Antibacterial parameters of the oils extracted from the adipose tissue of *Lutjanus dentatus* (mg/mL)

Bacteria			Ciprofloxacin			Drying at 45 °C						cooking at 95 °C					
Gram -		MIC (µg/mL)	Oil			Nanoemulsion			Oil			Nanoemulsion					
			MIC	MBC	R	MIC	MBC	r	MIC	MBC	R	MIC	MBC	r			
<i>Escherichia coli</i>	ATCC 8739	16	256	-	-	6.25	-	50	32	-	-	1.56	12.5	21			
	ECO20	8	128	-	-	6.25	-	20	128	-	-	6.25	-	20			
	ECOWDCM87	8	128	-	-	12.5	-	10	64	-	-	3.12	-	21			
	EC20	8	32	256	8	1.56	12.5	21	32	256	8	0.78	6.25	41			
<i>Enterobacter c.</i>	ENTB 62	8	128	-	-	12.5	-	10	64	128	2	3.12	-	21			
<i>Klebsiella pneumoniae</i>	KLPB 76	8	64	-	-	6.25	-	10	256	-	-	3.12	-	82			
	KLPB 77	16	32	128	4	1.56	12.5	21	128	-	-	3.12	-	41			
	KLPB 94	4	64	-	-	6.25	-	10	128	256	2	6.25	-	20			
	KLPB 95	16	64	-	-	3.12	-	21	256	-	-	6.25	-	50			
	KLPB 101	4	16	128	8	0.39	-	41	128	-	-	6.25	-	20			
	KLPB 91	2	64	-	-	6.25	-	10	256	-	-	6.25	-	50			
<i>Shigella flexneri</i>	SFB 93	2	64	-	-	6.25	-	10	256	-	-	3.12	-	82			
<i>Salmonella enterica serovar typhi</i>	SA ATCC 2593	32	256	-	-	3.12	-	82	128	-	-	3.12	-	41			
	SALH 46	2	128	-	-	-	Nd	Nd	128	-	-	6.25	-	20			
	SAL	4	-	Nd	-	3.12	128	Nd	256	-	-	3.12	-	82			
	Sal anatu	4	-	Nd	-	6.25	-	Nd	256	-	-	3.12	-	82			
<i>Citrobacter freundii</i>	CITB 126	16	128	-	-	3.12	-	41	256	-	-	6.25	-	50			
	CITB 80	16	32	128	4	1.56	12.5	21	16	128	8	6.25	-	3			
<i>Yersinia enterocolitica</i>	YERB 121	16	32	256	8	1.56	12.5	21	32	-	-	1.56	12.5	21			
	YERB 126	2>	64	-	-	1.56	-	41	128	-	-	3.12	-	41			
<b>Gram +</b> <i>Staphylococcus aureus</i>	STAB 74	32	64	-	-	0.39	12.5	164	128	-	-	6.25	-	20			
	MRSA 3	32	128	-	-	6.25	-	20	128	-	-	6.25	-	20			
	STAPH	8	64	256	4	6.25	12.5	10	32	128	4	3.12	-	10			

**MIC:** Minimal Inhibitory Concentration    **MBC:** Minimal Bactericidal Concentration    **Nd:** Not determined    **R:** MBC/MIC ratio    **r:** Oil MIC Nanoemulsion MIC ratio

### 3.5. Interactions of *L. dentatus* oils with some antibiotics

The combination of oils with antibiotics showed that the oil from the adipose tissue of *L. dentatus* obtained by drying at 45 °C potentiated the activity of Gentamicin on all the bacterial strains tested, that of Ciprofloxacin and Chloramphenicol on all the strains except *S. flexneri*. This oil also potentiated the activity of Amoxicillin on *E. coli*, *K. pneumoniae*, *S. typhi* and *C. freundii* and also of Tetracycline on *E. coli*, *E. cloacae*, *K. pneumoniae*, *S. typhi*, *C. freundii* and *S. aureus*. In contrast, the antagonistic and indifferent effects were observed with few bacterial strains (Table 4).

The oil obtained after cooking in general potentialized the activity of Amoxicilin, Ciprofloxacin, Chloramphenicol, Tetracyclin and Gentamycin on most of the bacterial strains used. This potentiative activity sometimes varied with the bacterial species. In addition, in certain cases, indifference and antagonism was selectively noted on some bacterial strains (Table 5).

### 3.6. Discussion

The cooking method made it possible to obtain the adipose tissue oil from *L. dentatus* with a better yield than with the 45 °C drying method. This increase could be attributed to the high temperature and pressurized steam which could favor the release of the oil from the tissues.

The variation in the acid index could be associated to the extraction method. Indeed, the extraction by drying at 45 °C was carried out for 24 hours, while the extraction after cooking at 95 °C was carried out for 20 min and at relatively high temperature. Furthermore, the low content of free fatty acids at 95 °C could be due to the deactivation of the lipolytic enzymes by heat, thus preventing hydrolysis in the cooked product. These results are in agreement with those obtained by Weber et al. (2008) according to who the cooking methods applied to catfish caused a significant reduction in its free fatty acid content. The acid index of the oil from the adipose tissue of *L. dentatus* extracted by both methods was in accordance with the standard ( $\leq 3$  mg KOH/g of oil) (CODEX ALIMENTARIUS, 2017).

A drop in the oil's iodine indicator is attributed to the destruction of fatty acid double bonds by oxidation, cleavage or polymerization (Tynek et al., 2001). The fatty tissue oil of *L. dentatus* obtained by drying at 45 °C had a lower iodine index than that obtained by pressing after cooking at 95 °C. The prolonged exposure of this oil to heat during extraction by drying at 45 °C leads to a deterioration of the double bonds. Indeed, the exposure of oil to heat leads to an alteration of the double bonds of unsaturated fatty acids (Eymard, 2003). Likewise, the peak at 3008  $\text{cm}^{-1}$ , linked to the CH stretching vibration of the cis double bond (=CH), which provides information on the degree of lipid unsaturation is greater in the oil obtained after cooking. A similar peak at 3010  $\text{cm}^{-1}$ , linked to the CH stretching vibration of the cis double bond (=CH), was obtained in the lipids of salmon (Guillen et al., 2004). In addition, the two peaks just below 3000 (2955-2922  $\text{cm}^{-1}$  and 2853-2849  $\text{cm}^{-1}$ ) attributed to the absorption caused by the asymmetrical and symmetrical stretching vibrations of the methyl and methylene groups could be linked to oxidation of oils with reduction of unsaturations.



**Table 4:** Interactions of the oil from the adipose tissue of *L. dentatus* obtained by drying at 45 ° C with some antibiotics depending on the bacterial strains

Bacteria		Ciprofloxacin		Amoxicillin		Tetracyclin		Gentamicin		Chloramphenicol						
		MIC	Association	MIC	Association	MIC	Association	MIC	Association	MIC	Association					
<b>Gram -</b>			<b>MIC</b>	<b>2MIC</b>	<b>MIC</b>	<b>2MIC</b>	<b>MIC</b>	<b>2MIC</b>	<b>MIC</b>	<b>2MIC</b>	<b>MIC</b>	<b>2MIC</b>		<b>MIC</b>	<b>2MIC</b>	
<i>Escherichia coli</i>	<b>ATCC 8739</b>	8	4(0.5) <sup>S</sup>	8(1) <sup>Ad</sup>	16	16(1) <sup>Ad</sup>	16(1) <sup>Ad</sup>	32	32(1) <sup>Ad</sup>	16(0.5) <sup>S</sup>	16	8(0.5) <sup>S</sup>	8(0.5) <sup>S</sup>	32	64(2) <sup>In</sup>	32(1) <sup>Ad</sup>
<i>Enterobacter cloacae</i>	<b>ENTB 62</b>	8	16(2) <sup>In</sup>	8(1) <sup>Ad</sup>	16	32(2) <sup>In</sup>	32(2) <sup>In</sup>	16	32(2) <sup>In</sup>	16(1) <sup>Ad</sup>	16	8(0.5) <sup>S</sup>	8(0.5) <sup>S</sup>	16	64(4) <sup>In</sup>	16(1) <sup>Ad</sup>
<i>Klebsiella pneumoniae</i>	<b>KLPB 95</b>	16	32(2) <sup>In</sup>	16(1) <sup>Ad</sup>	16	32(2) <sup>In</sup>	16(1) <sup>Ad</sup>	32	16(0,5) <sup>S</sup>	16(0,5) <sup>S</sup>	8	4(0.5) <sup>S</sup>	8(1) <sup>Ad</sup>	32	32(1) <sup>Ad</sup>	32(1) <sup>Ad</sup>
<i>Shigella flexneri</i>	<b>SFB 93</b>	2	16(8) <sup>An</sup>	8(4) <sup>In</sup>	16	32(2) <sup>In</sup>	32(2) <sup>In</sup>	16	32(2) <sup>In</sup>	32(2) <sup>In</sup>	8	8(1) <sup>Ad</sup>	16(2) <sup>In</sup>	2	16(8) <sup>An</sup>	16(8) <sup>An</sup>
<i>Salmonella enterica serovar typhi</i>	<b>SA ATCC 2593</b>	32	16(0.5) <sup>S</sup>	32(1) <sup>Ad</sup>	8	16(2) <sup>In</sup>	8(1) <sup>Ad</sup>	32	32(1) <sup>Ad</sup>	64(2) <sup>In</sup>	8	2(0.25) <sup>S</sup>	8(1) <sup>Ad</sup>	16	16(1) <sup>Ad</sup>	16(1) <sup>Ad</sup>
<i>Citrobacter freundii</i>	<b>CITB 126</b>	16	32(2) <sup>In</sup>	16(1) <sup>Ad</sup>	8	2(0.25) <sup>S</sup>	4(0.5) <sup>S</sup>	32	32(1) <sup>Ad</sup>	32(1) <sup>Ad</sup>	16	16(1) <sup>Ad</sup>	16(1) <sup>Ad</sup>	32	32(1) <sup>Ad</sup>	16(0.5) <sup>S</sup>
<i>Yersinia enterocolitica</i>	<b>YERB 121</b>	16	8(0.5) <sup>S</sup>	8(0,5) <sup>S</sup>	8	32(4) <sup>In</sup>	16(2) <sup>In</sup>	2	16(8) <sup>An</sup>	16(8) <sup>An</sup>	8	4(0.5) <sup>S</sup>	8(1) <sup>Ad</sup>	32	32(1) <sup>Ad</sup>	16(0.5) <sup>S</sup>
<b>Gram +</b>																
<i>Staphylococcus aureus</i>	<b>MRSA 3</b>	32	16(0.5) <sup>S</sup>	32(1) <sup>Ad</sup>	4	16(4) <sup>In</sup>	16(4) <sup>In</sup>	16	8(0.5) <sup>S</sup>	8(0.5) <sup>S</sup>	16	16(1) <sup>Ad</sup>	16(1) <sup>Ad</sup>	32	32(1) <sup>Ad</sup>	32(1) <sup>Ad</sup>

**MIC:** Minimal Inhibitory Concentration    A = Antagonism    S = Synergy    I = Indifference    Ad = Additive

**Table 5:** Interactions of the oil from the adipose tissue of *L. dentatus* obtained after cooking at 95 °C with some antibiotics depending on the bacterial strains

Bacteria	MIC	Ciprofloxacin		Amoxicillin		Tetracyclin		Gentamicin		Chloramphenicol						
		MIC	Association	MIC	Association	MIC	Association	MIC	Association	MIC	Association					
<b>Gram -</b>			<b>MIC</b>	<b>2MIC</b>	<b>MIC</b>	<b>2MIC</b>	<b>MIC</b>	<b>2MIC</b>	<b>MIC</b>	<b>2MIC</b>	<b>MIC</b>	<b>2MIC</b>				
<i>Escherichia coli</i>	ATCC 8739	8	16(2) <sup>In</sup>	16(2) <sup>In</sup>	16	16(1) <sup>Ad</sup>	16(1) <sup>Ad</sup>	32	8(0,25) <sup>S</sup>	8(0.25) <sup>S</sup>	16	32(2) <sup>In</sup>	32(2) <sup>In</sup>	32	32(1) <sup>Ad</sup>	64(2) <sup>In</sup>
<i>Enterobacter cloacae</i>	ENTB 62	8	32(4) <sup>In</sup>	16(2) <sup>In</sup>	16	64(4) <sup>In</sup>	32(2) <sup>In</sup>	16	8(0,5) <sup>S</sup>	16(1) <sup>Ad</sup>	16	16(1) <sup>Ad</sup>	8(0.5) <sup>S</sup>	16	8(0,5) <sup>S</sup>	8(0.5) <sup>S</sup>
<i>Klebsiella pneumoniae</i>	KLPB 95	16	16(1) <sup>Ad</sup>	16(1) <sup>Ad</sup>	16	16(1) <sup>Ad</sup>	8(0.5) <sup>S</sup>	32	16(0,5) <sup>S</sup>	16(0.5) <sup>S</sup>	8	32(4) <sup>In</sup>	2(0.25) <sup>S</sup>	32	32(2) <sup>In</sup>	16(1) <sup>Ad</sup>
<i>Shigella flexneri</i>	SFB 93	2	32(16) <sup>An</sup>	16(8) <sup>An</sup>	16	8(0.5) <sup>S</sup>	4(0.25) <sup>S</sup>	16	8(0,5) <sup>S</sup>	8(0.5) <sup>S</sup>	8	16(2) <sup>In</sup>	8(1) <sup>Ad</sup>	2	32(16) <sup>An</sup>	16(8) <sup>An</sup>
<i>Salmonella enterica serovar typhi</i>	SA ATCC 2593	32	64(2) <sup>In</sup>	16(0.5) <sup>S</sup>	8	32(4) <sup>In</sup>	32(4) <sup>In</sup>	32	64(2) <sup>In</sup>	32(1) <sup>Ad</sup>	8	64(8) <sup>An</sup>	32(4) <sup>In</sup>	16	32(2) <sup>In</sup>	32(2) <sup>In</sup>
<i>Citrobacter freundii</i>	CITB 126	16	32(2) <sup>In</sup>	32(2) <sup>In</sup>	8	32(4) <sup>In</sup>	32(4) <sup>In</sup>	32	64(2) <sup>In</sup>	32(1) <sup>Ad</sup>	16	32(2) <sup>In</sup>	16(1) <sup>Ad</sup>	32	32(2) <sup>In</sup>	32(1) <sup>Ad</sup>
<i>Yersinia enterocolitica</i>	YERB 121	16	32(2) <sup>In</sup>	32(2) <sup>In</sup>	8	8(1) <sup>Ad</sup>	8(1) <sup>Ad</sup>	2	32(16) <sup>An</sup>	32(16) <sup>An</sup>	8	64(8) <sup>An</sup>	32(4) <sup>In</sup>	32	32(1) <sup>Ad</sup>	16(0.5) <sup>S</sup>
<b>Gram +</b>																
<i>Staphylococcus aureus</i>	MRSA 3	32	32(1) <sup>Ad</sup>	32(1) <sup>Ad</sup>	4	32(8) <sup>An</sup>	32(8) <sup>An</sup>	16	32(2) <sup>In</sup>	32(2) <sup>In</sup>	16	16(1) <sup>Ad</sup>	16(1) <sup>Ad</sup>	32	32(1) <sup>Ad</sup>	32(1) <sup>Ad</sup>

MIC: Minimal Inhibitory Concentration    A = Antagonism    S = Synergy    I = Indifference    Ad = Additive

The high value of the peroxide index obtained in *L. dentatus* adipose oil obtained by drying at 45 °C compared to that obtained by pressing after cooking could be due to a prolonged reaction of oxygen with double bonds during drying at 45 °C. Furthermore, the peak associated with the hydroxyl groups formed during oxidation, appearing near 3350 cm<sup>-1</sup> is higher in the oil obtained by drying at 45 °C compared to that obtained after cooking. The decrease in the intensity of this band may suggest the decomposition of the hydroperoxide to give secondary lipid oxidation products due to heat.

The thiobarbituric acid index of oils from adipose tissue of *L. dentatus* obtained after cooking at 95 °C and drying are not significantly different ( $P > 0.05$ ). According to food standards, the oils were of good quality because they presented indices lower than 10 µmol MDA/kg (Weber et al., 2008). The anisidine index of oils from adipose tissue of *L. dentatus* obtained by drying (40,94±0,87) was significantly higher ( $P < 0.05$ ) compared to that obtained after cooking (37,11±0,36). Prolonged exposure to heat resulted in the transformation of unstable primary oxidation compounds (hydroperoxides) into stable secondary compounds (Cuvelier and Maillard, 2012).

According to Guillén and Cabo (Guillén and Cabo), the spectral regions between 1265 and 1014 cm<sup>-1</sup> (Figure 1d) are associated with the stretching vibration of the C-O ester group and the bending vibration of the C-O group of alcohols and ethers. They show a slight difference from one extraction method to another. Thus, the higher peaks in the oil obtained by drying at 45 °C may reflect a more advanced oxidation of the lipids. The results are in accordance with the oils indices.

The evaluation of the antibacterial activity of oils from adipose tissue of *L. dentatus* obtained by the two extraction methods revealed MICs values between 16 and 256 mg/mL, reflecting the activities of these oils on bacteria responsible of foodborne diseases. This antibacterial activity could be justified by the presence in these oils of saturated fatty acids and polyunsaturated fatty acids of the family of omega-3 (linoleic acid, eicosapentanoic acid, docosahexanoic acid) and omega-6 (linolenic acid, arachidonic acid) (Chitra and Radhakrishnan, 2011). These oils could therefore be used to relieve stomachaches due to contamination by these enterobacteria.

The oil obtained following cooking was active on a wide range of bacteria compared to that obtained after drying at 45 °C. This could be due to the modification of the chemical composition reflected by the quality indices of the oil obtained after drying at 45 °C which was more affected during the extraction.

The antibacterial activity of the emulsions from *L. dentatus* adipose tissue oils was greater compared to oils used for their preparation. This can be explained by the fact that, the emulsions improve the penetration of the active fatty acids through the bacterial cell membrane thanks to their large specific surface and to the reduction of the interfacial tension of the droplets (Chime et al., 2014). Indeed, in comparison with macroemulsions, nanoemulsions in question in this work have better solubility and much greater specific surface, which gives an optimized diffusibility of the active substance.

Oils rich in polyunsaturated fatty acids behave as adjuvants that can modulate the activity of certain antibiotics (Desbois and Smith, 2010). The oils from *L. dentatus* potentiated the activity of Ciprofloxacin, Amoxicilin, Tetracycline, Gentamicin and Chloramphenicol on the bacteria tested with a better potentiation on the strains of *Escherichia coli* *Klebsiella pneumoniae*. The potentiating activity is explained by the presence of PUFAs including omega 3 and omega 6, saturated fatty acids (myristic and palmitic) and oxidation products of fatty acids which could improve the activity of antibiotics.

#### 4. CONCLUSION

This study shows that the extraction yield as well as the quality indices vary depending on the extraction method. 45 °C and 95 °C exudation affected the quality of this oil, however the oil extracted by 45 °C exudation was most affected during extraction. The oil extracted from the adipose tissue of *Lutjanus dentatus* exhibited general antibacterial activities. However, the oil exudated at 95 °C was the most active on a wide range of bacteria. Nanoemulsions exhibit better antibacterial activity compared to those of the oils used for their formulation. *L. dentatus* adipose tissue oil potentiates the activity of Ciprofloxacin, Amoxicilin, Tetracycline, Gentamycin and Chloramphenicol on certain bacteria responsible for food poisoning.

The antibacterial activity of oil from *L. dentatus* adipose tissue is of particular interest for pharmaceutical industries. Thus, this oil could help in the treatment of foodborne infections or used as food supplements to improve the effectiveness of some antibiotics.

#### 5. CONFLICTS OF INTEREST

None.

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