WILEY

Research Article

Effects of Storage Temperature and Spices Incorporation on the Stability and Antibacterial Properties of *Fontitrygon margarita* (Günther, 1870) Liver Oil

Boris Simo Noutsa ⬤,[1] **Arlette Danelle Deutchoua Djitieu** ⬤,[1,2]
Fabrice Fabien Dongho Dongmo ⬤,[3] **Fabrice Hervé Njike Ngamga** ⬤,[4]
Sammuel Raymond Tchabong ⬤,[1] **Ousman Tamgue** ⬤,[3] **Rosalie Anne Ngono Ngane** ⬤,[3]
and François Tchoumbougnang[1]

[1]*Department of Processing and Quality Control of Aquatic Products, Institute of Fisheries and Aquatic Sciences, University of Douala, P.O. Box 7236, Douala, Cameroon*
[2]*Laboratory of Analytical Chemistry, Faculty of Sciences, University of Yaoundé I, B. P. 812, Yaoundé, Cameroon*
[3]*Department of Biochemistry, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon*
[4]*Research Unit of Biochemistry of Medicinal Plants, Food Sciences and Nutrition, University of Dschang, P.O. Box 67, Dschang, Cameroon*

Correspondence should be addressed to Fabrice Fabien Dongho Dongmo; donghofabrice@gmail.com

Fontitrygon margarita liver oil, rich in unsaturated fatty acids, is susceptible to oxidation during storage, which can diminish its antibacterial qualities. This study examines the effects of storage temperature and the addition of spices on the stability and antibacterial properties of *F. margarita* liver oil. Oils with added spices were stored in opaque bottles at room temperature ($28 \pm 2°C$) and in a refrigerator ($4°C$) and were periodically analyzed over a six-month period. Standard methods were used to determine oil quality indices; the Fourier transform infrared (FTIR) profile was assessed by spectroscopy; and antibacterial activities were measured using the broth microdilution method. The quality indices, FTIR profile, and antibacterial activities of the oil were evaluated and compared based on the incorporation of spices. The quality indices of oil extracted without a stabilizer and stored at room temperature significantly increased over time. The antibacterial activity of these oils gradually decreased during storage, with the minimal inhibitory concentrations (MICs) on bacterial strains of *Escherichia coli* (EC 137), *Enterobacter cloacae* (ENT 119 and ENT 51), and *Yersinia enterocolitica* (YERB 1) increasing from 16 to 128 mg/ml. Regardless of oil quality indices, oils stored in a refrigerator had lower values and better antibacterial activities than those stored at room temperature (($16 \leq MIC \leq 64$ mg/ml on the strains of EC 137, YERB 1, ENT 51, and *Klebsiella pneumoniae* (KL 11)). The inclusion of spices significantly reduced the oxidative reaction in the oils and maintained the antibacterial activities of the tested oils. Given its antibacterial properties, *F. margarita* liver oil holds significant potential for the nutraceutical industry and could be used as a dietary supplement. This research underscores the importance of proper storage conditions and the use of natural stabilizers in maintaining the quality of such valuable natural resources.

1. Introduction

Omega-3 fatty acids, especially long-chain polyunsaturated fatty acids (PUFAs) from fish oil, have been the focus of much research in recent years. The global fish oil market was

projected to reach a value of USD 4.08 billion by 2022, spurred by a rise in aquaculture activities and increased consumer awareness of the health benefits of omega-3 PUFAs, including eicosapentaenoic acid (EPA; $C20: 5n-3$) and docosahexaenoic acid (DHA; $22:6n-3$). EPA

and DHA are associated with potential health benefits such as the prevention of coronary artery diseases, hypertension, diabetes, arthritis, autoimmune disorders, neurological diseases, cancer, improved brain development, and eye function in infants [1]. They exhibit a range of biological activities, including anti-inflammatory, antioxidant, anti-obesity, neuroprotective, and antimicrobial activities [2, 3]. PUFAs possess a unique capability to integrate with microbial cell membranes, thereby substantially increasing membrane fluidity. This alteration can trigger the opening of permeability channels, disrupt crucial concentration gradients, and ultimately lead to the death of microbial cells [4, 5]. In addition to influencing membrane fluidity, PUFAs demonstrate a range of antimicrobial activities. They have the ability to hinder microbial metabolism, inhibit growth, and thwart the formation of biofilms, which serve as a microorganism's shield against external threats. PUFAs also bolster the host's immune defenses and interrupt microbial communication, a process known as quorum sensing. Furthermore, PUFAs can induce oxidative stress in microorganisms, causing cellular damage and death. These varied mechanisms underscore the potential of PUFAs as antimicrobial agents. Current research is exploring these effects in depth, with the aim of harnessing PUFAs to combat microbial infections [5–7].

Numerous research studies conducted in Cameroon have uncovered that certain fish species, particularly those inhabiting the regions of Yabassi and Youpwe, Douala, are capable of producing oils rich in long-chain PUFAs, such as EPA and DHA. These oils have also been found to possess antibacterial properties. A recent review by Dongho et al. [8] highlighted that, as of March 2023, numerous studies have confirmed the antibacterial efficacy of oils derived from four Cameroonian fish species: *Chrysichthys nigrodigitatus*, *Fontitrygon margarita*, *Hepsetus odoe*, and *Lutjanus dentatus*. Furthermore, a subsequent study by Zokou et al. [9] has showcased the antibacterial potential of oil from *Oreochromis niloticus*. Among these, the oil obtained from the liver of *F. margarita*, a common species in Youpwe, Douala, stands out due to its high oil extraction yield of 14.49 to 16.90% as shown by Noutsa et al. [2]. They also noted that this oil is rich in PUFAs, accounting for 28.52% of its composition, and includes EPA, DHA, linolenic acid (C18 : 3), arachidonic acid (C20 : 4), and oleic acid (C18 : 1). Moreover, it has been found to be effective against both Gram-positive and Gram-negative bacteria, which are often responsible for food poisoning. Interestingly, oils extracted through exudation have shown higher activity than those obtained by cooking and pressing.

Indeed, the high content of unsaturated fatty acids in fish oil makes it susceptible to oxidation. This process can lead to the formation of various compounds such as peroxides, aldehydes, and ketones [10]. Lipid oxidation is a complex series of reactions that occur in the presence of oxygen, resulting in a state commonly referred to as rancidity. This is one of the primary factors contributing to the deterioration of oil quality. The consequences of lipid oxidation are manifold. It can decrease the nutritional and market value of the oil, alter its taste, and modify its texture and appearance [11]. Furthermore, lipid oxidation can reduce the shelf life of the oil and limit its therapeutic benefits. This presents a significant challenge for the use of ω-3 PUFAs in food and pharmaceutical applications. Therefore, it is crucial to find effective ways to prevent or slow down the oxidation process to preserve the quality and benefits of these oils.

To ensure the sustainable use of *F. margarita* liver oil, it is essential to establish effective storage strategies that reduce oxidation and improve the longevity and quality of the oil [12]. Synthetic antioxidants such as butylhydroxytoluene (BHT) and citric acid have traditionally been the industry standard due to their proven effectiveness [12, 13]. However, the trend towards natural products has led to an increased demand for natural antioxidants such as tocopherols, which are found in a variety of foods [13, 14]. The high metal content in fish oil can potentially compromise the effectiveness of these antioxidants [15], prompting the investigation of alternative plant extracts such as rosemary, oregano, and tea. These extracts are rich in phenolic compounds and act as potent antioxidants that neutralize free radicals, chelate metals, or counteract singlet oxygen [12, 16]. The application of these antioxidants, either directly or through marination, requires careful management of concentrations to maintain the oil's flavor and aroma [12, 13]. It is crucial to maintain low temperatures during extraction and process quickly. After extraction, the oil's resistance to oxidation can be enhanced by promptly adding antioxidants, cooling rapidly, and employing techniques such as emulsification and encapsulation [12, 14]. Nano-emulsion has been demonstrated to enhance the preservation and antibacterial properties of *F. margarita* oil [2].

The stability of fish oil is influenced by storage duration and temperature. Appropriate storage postprocessing is vital, with unopened oils lasting 1–2 years and opened oils remaining fresh for 3–8 months if refrigerated [17, 18]. Storing away from heat, moisture, and light, particularly in a refrigerator (0–4°C), extends shelf life and slows down spoilage [12]. These practices ensure the maintenance of high-quality fish oil, thereby preserving its health benefits. Low-temperature storage enhances the fatty acid profile and physical properties of fish lipids, and the addition of antioxidants and plant extracts at low temperatures further improves lipid quality [12]. However, to the best of our knowledge, no existing studies have yet investigated the impact of incorporating antioxidants and varying storage conditions on the biological attributes of fish oils, especially their antimicrobial properties.

To further enhance the biological properties, particularly the antimicrobial properties, of oil extracted from the liver of *F. margarita* and, more generally, fish oils in Cameroon, it is crucial to devise strategies for their sustainable use. While refrigeration is the optimal preservation method, it is not a viable option for many in Cameroon due to its high cost and frequent power outages, especially in rural areas lacking electricity [19]. This underscores the need for supplementary methods that can augment refrigeration, such as the incorporation of natural antioxidants prior to extraction. This strategy would shield the oil from oxidation during the extraction process and subsequent storage at room

temperatures, and it would boost protection during storage at low temperatures. When identifying potential sources of natural antioxidants, dietary spices and herbs stand out for their rich phytocompounds with antioxidant properties, including phenolic compounds [20, 21]. In fact, Cameroon is renowned for its rich variety of herbs and spices, notably *Allium sativum*, *Piper nigrum*, and *Monodora myristica*. These species are not only prevalent but also extensively used [22, 23]. They are distinguished for their abundant phenolic compounds and flavonoids. Research has revealed that these spices contain phenolic compounds known for their anti-oxidant and antimicrobial properties. This includes phenolic acids such as caffeic, chlorogenic, cinnamic, p-coumaric, and syringic acids, along with flavonoids such as catechin, kaempferol, and quercetin. Not to be overlooked are other phenolic compounds with beneficial properties, such as elemicin in *M. myristica* and piperine in *P. nigrum* [24, 25]. Additionally, *A. sativum*, like other members of the Liliaceae family, contains sulfur compounds such as allyl trisulfide, allicin, diallyl disulfide, and diallyl sulfide, which are rec-ognized for their antioxidant and antimicrobial activities [26]. The antioxidant and antimicrobial properties of these spices are well-established in Cameroon [23, 27]. Further-more, their extracts and powders have proven to be potent antioxidants, stabilizing crude soybean oil during extraction and throughout accelerated storage [27]. However, the potential of these spices to mitigate lipid oxidation in fish oil and their impact on the antibacterial activities of fish oil warrant further investigation.

The primary aim of this study is to investigate the impact of storage temperature and the addition of *A. sativum*, *P. nigrum*, and *M. myristica* on the stability and antibacterial properties of *F. margarita* liver oil. The study will specifically target bacteria that cause food-borne illnesses.

2. Materials and Methods

2.1. Materials

2.1.1. Collection of Livers of Fontitrygon margarita. The livers were sourced from freshly caught samples of *F. margarita* fish at the Youpwe market, Douala, in April 2020. Throughout the month, we conducted three distinct collection drives. Skilled fish cleaners meticulously removed the livers from the fish's abdominal region. To maintain optimal freshness and quality, the livers were promptly placed in iceboxes for transport to the Institute of Fisheries and Aquatic Sciences' Valorization and Quality Control Laboratory at the University of Douala in Yabassi, Came-roon. The time from the market to the laboratory was 2 to 3 hours. This procedure was consistently replicated across all three collection campaigns, thereby ensuring three replicates for each experimental condition.

2.1.2. Spices. The bulbs of *A. sativum*, seeds of *P. nigrum*, and hulls of *M. myristica* used in this research were sourced from the local Youpwe market. These raw materials underwent

a meticulous drying process before being finely ground into powder using a traditional stone grinder. A small portion of these powders was then subjected to aqueous extraction to quantify the total phenolic and favonoid contents, which are key constituents contributing to the spices' potent antioxidant properties. The remaining powder was carefully stored for subsequent extraction procedures.

2.1.3. Bacteria. The study used several bacteria, including two reference strains of *Escherichia coli* (ATCC 10536) and *Salmonella enterica serovar typhi* (ATCC 28579). These were sourced from the Laboratory of Microbiology and Anti-microbial Substances at the University of Dschang in Cameroon. Additionally, clinical strains of *Escherichia coli* (EC 137), *Enterobacter cloacae* (ENT 119 and ENT 51), *Klebsiella pneumoniae* (KL 11), *Salmonella enterica serovar typhi* (SAL 9), *Citrobacter freundii* (CITB 81), *Yersinia enterocolitica* (YERB 1), and *Staphylococcus aureus* (ST 120) were obtained from the Laboratory of Biochemistry at the University of Douala.

2.2. Methods

2.2.1. Aqueous Extraction of Spices and Determination of Total Phenolic and Flavonoid Contents. Approximately 25 g of each spice powder was combined with sterilized distilled water to yield a 100 ml solution, thus creating an aqueous extract with a concentration of 25% w/v. This concoction was allowed to stand at room temperature ($28 \pm 2°C$) for 48 hours in a sterile flask, with regular shaking. The solution was then centrifuged (3000 rpm, 10 min) and filtered through a Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator set to a temperature range of 55–60°C.

The extracts were subsequently evaluated for their total phenolic, flavonoid, and tannin contents. The total phenolic content (TPC) was determined using the Folin–Ciocalteu method, as described by Womeni et al. [27]. In this pro-cedure, $20 \mu L$ of the appropriately diluted spice extract (100 mg/ml) was combined with 0.2 mL of Folin–Ciocalteu reagent and 2 mL of distilled water in a test tube. This mixture was incubated at room temperature for 3 minutes. Subsequently, 1 mL of a 20% sodium carbonate solution was added, and the mixture was re-incubated at room tem-perature for an additional 2 hours. A blank was prepared using $400 \mu L$ of distilled water in place of the sample. The absorbance of the resultant blue mixture was measured at 765 nm using a quartz cuvette. Gallic acid was used as the standard, and the TPC was quantified as mg of gallic acid equivalents (mgGAE/g).

The total flavonoid content of the extracts was de-termined using an assay that forms a complex with alu-minum chloride [28]. The process began by mixing $100 \mu l$ of each diluted extract (100 mg/ml) with $100 \mu l$ of 5% sodium nitrate. This mixture was allowed to stand for 6 minutes. Subsequently, $150 \mu l$ of a 10% aluminum chloride solution was added and the solution was left to stand for an additional

5 minutes. Following this, a 200 μl solution of 1 M sodium hydroxide was added sequentially. The absorbance of the resulting reaction mixture was measured at 510 nm using a UV spectrophotometer. The total flavonoid content of the extracts was then calculated in terms of quercetin equivalents (expressed as mgQE/g).

All these procedures were performed in triplicate to ensure the accuracy and reliability of the results.

2.2.2. Fish Oil Extraction and Storage.

After each collection campaign, the harvested *F. margarita* livers were meticulously sliced, amalgamated, and subsequently divided into five uniform batches for different extraction processes, some including stabilizers and some without. The first group was processed without stabilizers, acting as the control. The second group incorporated a common antioxidant, BHT, while the final three other groups were treated with various spices. The procedure was according to the protocol established by Womeni et al. [27]. Thus, the BHT was introduced into the liver samples at the legal limit of 200 ppm, and the spices, *A. sativum*, *P. nigrum*, and *M. myristic*, were each added at 2000 ppm. Then, the mixture was vigorously stirred for about 10 minutes to ensure the homogeneous diffusion of the antioxidants, and the oil extraction directly followed.

This extraction was performed using the exudation technique, following the methodology established by Takahashi and Mitsui as used by Bonilla and Hoyos-Concha [29]. Briefly, the samples were heated in a dry oven at 95°C for 10 minutes, after which the oil was collected. Anhydrous sodium sulfate (Na_2SO_4) was used to eliminate any traces of moisture from the oil. Immediately after extraction, each oil sample underwent physicochemical characterization (including quality index and Fourier transform infrared/FTIR profile) and the determination of antibacterial activities using standardized methods.

Each sample was then divided into two parts, stored in 50 mL opaque bottles, and kept for 180 days under different conditions—one portion in a refrigerator (4°C) and the other at room temperature (28 ± 2°C). Every 60 days of storage, the samples were analyzed for quality index and antibacterial activities. Additionally, the FTIR profile was analyzed only for the control sample stored at room temperature.

2.2.3. Chemical Characterization.

The chemical characterization of oil involves assessing quality indicators and the Fourier transform infrared (FTIR) spectrum to ensure its freshness, purity, and suitability for use. The acid value measures free fatty acid levels to gauge freshness, while the iodine value indicates unsaturation and potential oxidation. Peroxide value points to initial oxidation stages, and both thiobarbituric acid reactive substances (TBARS) and anisidine values assess secondary oxidation products. The total oxidation value (TOTOX) offers a comprehensive view of oxidative stability. Meanwhile, the FTIR spectrum provides insights into the oil's chemical structure by identifying functional groups.

(1) Fish Oil Quality Indices. The critical quality parameters of oil including acid, iodine, peroxide, TBARS, anisidine, and TOTOX values were analyzed following the standard methods endorsed by the Official Methods and Recommended Practices of the AOCS [30], the International Union of Pure and Applied Chemistry as mentioned by Paquot [31], and the International Dairy Federation quoted by Zhang et al. [32].

For acid value assessment, a sample of 1 g of oil (m) was placed into a beaker containing 100 mL of ethanol at 95°C. To this mixture, two drops of 0.1 N phenolphthalein solution were added as an indicator. The solution was then titrated with 0.1 N (T) potassium hydroxide (KOH) until a color change was observed, indicating the endpoint of the titration. The volume (V_1) of KOH required to reach this endpoint was recorded. A blank titration was also performed under the same conditions without the oil sample to account for any background reactions. The volume (V_0) of KOH used in the blank titration was noted. The acid value (mgKOH/g oil) is calculated using the following formula:

$$\text{Acid value} = \frac{V_1 - V_0}{m} \times 56,1 \times T. \tag{1}$$

As for iodine value determination, a 0.2 g sample of oil (m) was accurately weighed and transferred into a flask. To this, 15 mL of 80% carbon tetrachloride (CCl_4) solution and 25 mL of Wijs reagent were added. The flask was then hermetically sealed, gently agitated, and stored in a dark location for 1 hour to allow the reaction to proceed. Following the incubation period, 20 mL of 10% aqueous potassium iodide (KI) solution, 15 mL of distilled water, and 5 drops of 1% starch solution were introduced to the mixture. The resulting solution was titrated with 0.1 N (T) sodium thiosulfate until a color change indicated the endpoint. The volume of sodium thiosulfate used in the titration (V_1) was recorded. A blank titration was also conducted, following the same steps but without the oil sample, to measure the background volume (V_0) of sodium thiosulfate. The iodine value (g I_2/100 g oil), which indicates the amount of iodine absorbed by the fats and oils in the sample, was calculated using the following formula:

$$\text{Iodine value} = \frac{V_0 - V_1}{m} \times 12,69 \times T. \tag{2}$$

Concerning peroxide value assessment, in a clean glass tube, 50 mg of the oil sample was introduced. To this, 9.8 mL of a chloroform-methanol mixture in a 7 : 3 ratio was added, and the contents were thoroughly mixed. Next, 50 μL of a 30% ammonium thiocyanate solution was added, followed by another round of mixing using a vortex mixer for 2–4 seconds. This was immediately followed by the addition

of 50 μL of an iron (II) chloride solution. The test tube was then vortexed again for 2–4 seconds to ensure complete mixing. The test tube was left to incubate at room temperature for 5 minutes. After incubation, the absorbance of the reaction mixture was measured at 500 nm, against a blank that contained all the reactants except the oil sample. The entire procedure was conducted in a dimly lit environment and completed within 8 minutes for each test to prevent light-induced changes. The peroxide value (mEqO$_2$/kg oil) was calculated from the ferric ion (Fe^{3+}) calibration curve using the following formula:

$$\text{Peroxide value} = \frac{(\text{As} - \text{Ab}) \times y}{55,84 \times m}, \tag{3}$$

where As is the absorbance of the sample; Ab is the absorbance of the blank; y is the slope obtained from the calibration curve (with a value of 38.46); m is the mass of the oil sample in g; and 55.84 is the molar mass of iron.

Regarding the TBARS assay, a 1 g sample of oil was dissolved in 10 mL of carbon tetrachloride (CCl$_4$). To this solution, 10 mL of 0.67% thiobarbituric acid (TBA) solution and an equal volume of glacial acetic acid were added. The mixture was stirred intermittently over 2 hours and subsequently centrifuged at 1000 rpm for 5 minutes. The aqueous phase was carefully separated and subjected to incubation in boiling water for 1 hour. After incubation, the absorbance of the solution was measured at 532 nm. The concentration of TBARS (μmol MDA/Kg oil) was calculated using the following formula:

$$\text{Thiobarbituric acid value} = \frac{\text{Abs}}{d \times m}, \tag{4}$$

where Abs is the absorbance reading at 532 nm; d is the path length of the curve (tank thickness); and m is the mass of the oil sample in g.

To determine the anisidine value, 0.5 g of oil were dissolved in a 25 mL volumetric flask. The volume was then made up to the mark with isooctane. Then, 5 mL of the above-prepared solution (oil and isooctane) was transferred into a test tube. To this test tube, 1 mL of p-anisidine (0.25 % in glacial acetic acid) was added. The mixture in the test tube was well stirred and then left to stand in the dark for 10 minutes to react. The absorbance of the solution was measured at 350 nm. A blank test was also performed using the same procedure but without the oil sample. The anisidine value was calculated using the following formula:

$$\text{Anisidine value} = \frac{25 \times (1, 2 \times \text{As} - \text{Ab})}{m}, \tag{5}$$

where As is the absorbance of the lipid solution after reaction with anisidine; Ab is the absorbance of the lipid solution (blank); and m is the mass of the oil sample in g.

The TOTOX was determined using the equation: TOTOX = 2 × peroxide value + anisidine value. This formula quantifies the overall extent of oil oxidation by combining the peroxide value, which measures primary oxidation products, with the anisidine value, indicating secondary oxidation products.

(2) Fourier Transform Infrared Spectra Analysis. Infrared (IR) spectra ranging from 3800 to 500 cm^{-1} were captured using a tensor 27 (Bruker, Wissembourg, France). This device was paired with an ATR prism crystal accessory and an MCT (mercury cadmium telluride) detector. The resolution of the spectra was set at 4 cm^{-1}. The measurements were conducted at room temperature using approximately 2 μL of fish oils. These oils 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 then subtracted using the OPUS version 6.3.2 spectrum software (PerkinElmer Inc.).

2.2.4. Antibacterial Activity.

The antibacterial properties of the oil were assessed using the broth microdilution method in 96-well microplates. This method is in accordance with the standards set by the Clinical and Laboratory Standards Institute [33].

(1) Preparation of Stock Solutions of Oils and Antibiotic. The oils were prepared as stock solutions at a concentration of 1024 mg/mL in a 5% solution of Tween 80. Additionally, the antibiotic ciprofloxacin was prepared at a concentration of 256 μg/mL.

(2) Inoculum Preparation. Muller–Hinton agar culture was used to prepare the bacterial strain's inoculum. Bacterial suspensions were created with a concentration of approximately 1.5×10^8 CFU/mL, equivalent to McFarland turbidity standard no. 0.5. The inoculum was then obtained by diluting this suspension 100 times, resulting in a final concentration of 1.5×10^6 CFU/mL.

(3) Antibacterial Activity Evaluation of Fish Oils. Each well of the microplate was filled with 100 μL of culture broth (MHB). Then, 100 μL of oil was added to the top wells, and a series of twofold dilutions were performed to achieve a final concentration ranging from 2 to 256 mg/mL in a total volume of 100 μL/well. Each well was further diluted with 100 μL of inoculum. The plates were then incubated at 35°C for 18 hours. Growth was monitored using p-iodotetrazolium chloride (0.2 mg/mL) oil [34]. Viable bacteria changed the yellow dye of iodonitrotetrazolium into a pink color. The lowest concentration of oil, at which no visible color change was noted, was considered as the minimum inhibitory concentration (MIC). Ciprofloxacin was used as a positive control in this experiment.

2.3. Data Handling and Statistical Analyses.

The area under the curve (AUC) from day 0 to day 180 was calculated for each parameter, for both quality indices and antibacterial activity, under each storage condition and treatment. The value at day 0 was used as the baseline. The AUC calculation was used to compare each parameter and the effects of storage temperature and different stabilizers.

The results were presented as the mean ± standard deviation. The statistical significance was determined using Student's *t*-test and one-way analysis of variance (ANOVA),

followed by Turkey's post hoc tests for pairwise separation and comparison of means. The statistical analysis was performed using GraphPad Prism software version 5.9 (GraphPad Software; La Jolla, California, USA).

3. Results and Discussion

The phenolic content of the spices, determined through the Folin–Ciocalteu method relative to the dry extract, was found to be 25.67 ± 1.01 mgGAE/g for *A. sativum*, 21.37 ± 0.98 mgGAE/g for *P. nigrum*, and 22.5 ± 0.51 mgGAE/g for *M. myristica*. Regarding flavonoid levels, *A. sativum* exhibited 5.88 ± 0.24 mgQE/g, *P. nigrum* exhibited 6.71 ± 0.21 mgQE/g, and *M. myristica* exhibited 6.56 ± 0.51 mgQE/g. The presence of these phenolic and flavonoid compounds underscores the potential of these spices as dietary antioxidants. Generally, the greater the concentration of these compounds, the stronger the antioxidant capacity is expected to be [25]. Of the three, *A. sativum* boasts the highest phenolic content, suggesting it may have the strongest antioxidant effects. Although *A. sativum* presents the lowest flavonoid concentration, both *P. nigrum* and *M. myristica* exhibit higher levels, potentially amplifying their antioxidant efficacy. This evidence further validates the selection in this study of these three spices as natural antioxidants, offering protection against the degradation of fish oil quality during extraction processes and storage. Additionally, the inclusion of these compounds in the extracted oil is likely to enhance its antibacterial properties [23, 25].

Tables 1 and 2 provide a comprehensive overview of the quality indices and antibacterial properties of *F. margarita* liver oil, respectively. These tables detail how these characteristics change over time, with different storage temperatures and stabilizers. Table 1 includes quality indices such as acid value, iodine value, thiobarbituric acid value, peroxide value, anisidine value, and TOTOX. These indices are crucial for assessing the quality and stability of the oil. However, Table 2 presents the antibacterial properties of the oil. This table shows how these properties vary with storage time, temperature, and the use of different stabilizers.

3.1. Effect of Stabilizers on Properties of Fontitrygon margarita Liver Oil during Extraction

3.1.1. Physicochemical Parameters. As indicated in Table 1, the acid, iodine, peroxide, anisidine values, and total oxidation of the extracted oil (day 0) did not significantly differ compared with the control, oil extracted with BHT, and those extracted with spices. Similarly, there were no significant differences between the spices. However, these oil samples remain compliant with the 2021 Codex Alimentarius Commission [35]. This could be attributed to the limited time of exposure of the oil to heat (10 min), thus limiting the reaction of oxygen with double bonds. Furthermore, the infrared spectroscopy conducted between 3800 and 500 cm^{-1} to identify the functional groups present in *F. margarita* liver oil after extraction (Figure 1) revealed similar spectra of whether the oil was extracted with

stabilizers or not. The five spectra had the same shape and intensity, as they originated from the same fish. This suggests that the nature and content of the chemical compounds present in the oil are relatively the same, which is consistent with the quality index results. Generally, the FTIR spectrum exhibited similar regions of functional group vibrations as reported previously for *Fontitrygon margarita* liver oils extracted without stabilizer oil [2].

3.1.2. Antibacterial Properties. As observed in Table 1, oil samples extracted with a stabilizer (spices or BHT) exhibited antibacterial activity immediately after extraction, compared with the control sample, regardless of the bacterial strain. Similarly, irrespective of the presence of stabilizers, the antibacterial activity of the different oil samples had a MIC between 16 mg/mL and 32 mg/mL for all the bacterial strains tested. This reflects the activities of these oils against bacteria responsible for food-borne diseases. For all these oil samples, the best antibacterial activity (MIC = 16 mg/mL) was obtained on the bacterial strain *Yersinia enterocolitica* (YERB 1), while the lowest antibacterial activity (MIC = 32 mg/mL) was obtained on the bacterial strain *Citrobacter freundii* (CITB 81). The antibacterial properties observed may be attributed to the presence of both saturated and PUFAs in the oil samples, notably the ω-3 family, in which EPA and DHA, along with the ω-6 family comprising linolenic acid and arachidonic acid, have been identified as key contributors. These fatty acids, as characterized in the fatty acid profiles from our previous research [2], are known for their potent antimicrobial activities. The harmful impact of these fatty acids on bacterial cells is due to their surfactant nature, which facilitates their interaction with the cell membrane, leading to the formation of temporary or permanent pores of varying sizes. This interaction increases the membrane's permeability and fluidity, causing the leakage of cellular contents, which may result in growth inhibition, cell lysis, or cellular death. Moreover, saturated fatty acids have been shown to trigger autolysis in bacterial cell walls for certain species, attributed to a decrease in membrane fluidity [4, 5].

3.2. Effect of Storage at Room Temperature on Properties of Fontitrygon margarita Liver Oil

3.2.1. Physicochemical Parameters. As indicated in Table 1, the acid, peroxide, thiobarbituric acid, and anisidine values, as well as the total oxidation of *F. margarita* liver oil extracted without a stabilizer (control) and stored at room temperature, significantly increased with storage time. This suggests a deterioration in the quality of fish oil during storage at room temperature. In fact, after 120 and 180 days of storage, respectively, the peroxide (6.11 ± 0.8 mEqO$_2$/kg) and thiobarbituric acid (10.54 ± 0.30 μmol MDA/Kg) values obtained from the oil were not compliant with the 2021 Codex Alimentarius Commission Standard guidelines [35]. However, the anisidine index (13.08 ± 1.11) remained within the standard limits even at the 180th day of storage. This deterioration in the quality of the oil is mainly due to its exposure at room temperature, as heat destabilizes the C=C

TABLE 1: Effect of spices and temperature storage on the quality indices of *Fontitrygon margarita* liver oil.

Parameters	Treatment	Standard	Day 0	Room temperature (28 ± 2°C)			Storage — Refrigeration (4°C)		
				Day 60	Day 120	Day 180	Day 60	Day 120	Day 180
Acid value (mgKOH/g)	Control		2.15 ± 0.84	3.15 ± 0.04*	4.64 ± 0.65***	5.32 ± 0.20***	2.51 ± 0.03	2.87 ± 0.23	3.72 ± 0.10*
	BHT		1.68 ± 0.17	2.52 ± 0.40*	2.80 ± 0.50**	3.62 ± 0.50***	2.22 ± 0.20*	2.45 ± 0.14*	2.86 ± 0.21**
	A. sativum	≤3	1.82 ± 0.21	3.04 ± 0.02**	3.73 ± 0.32***	3.95 ± 0.41***	2.38 ± 0.39	2.78 ± 0.43*	3.20 ± 0.12**
	P. nigrum		1.95 ± 0.32	3.12 ± 0.50**	3.51 ± 0.27**	3.75 ± 0.20**	2.30 ± 0.40	2.81 ± 0.05*	3.56 ± 0.14**
	M. myristica		2.05 ± 0.41	3.40 ± 0.26*	3.63 ± 0.26*	4.01 ± 0.74**	2.39 ± 0.13	2.71 ± 0.05*	3.62 ± 0.63**
Iodine value (gI$_2$/100 g)	Control		106.65 ± 5.00	104.55 ± 1.55	101.53 ± 1.66	95.12 ± 2.06*	104.52 ± 2.69	100.81 ± 2.39	98.12 ± 3.10
	BHT		108.71 ± 4.30	104.27 ± 2.80	100.44 ± 3.90	98.21 ± 3.10*	105.04 ± 3.20	104.30 ± 2.80	103.12 ± 2.50
	A. sativum	—	107.50 ± 6.10	103.70 ± 2.90	101.70 ± 4.01	97.35 ± 2.90*	104.20 ± 3.30	102.20 ± 4.02	100.54 ± 3.20
	P. nigrum		108.35 ± 5.20	105.35 ± 2.9	103.21 ± 3.10	99.75 ± 3.20	106.12 ± 3.50	104.12 ± 2.50	100.22 ± 4.50
	M. myristica		106.86 ± 3.70	104.62 ± 3.30	100.44 ± 3.90	95.10 ± 1.01*	104.61 ± 4.70	101.30 ± 2.80	99.33 ± 1.40
Thiobarbituric acid value (μmol MDA/Kg)	Control		3.20 ± 0.14	4.77 ± 0.27*	7.94 ± 0.32*	10.54 ± 0.30***	5.13 ± 0.59*	6.64 ± 0.28**	8.16 ± 0.27***
	BHT		2.93 ± 0.25	4.60 ± 0.88*	6.02 ± 0.15**	7.32 ± 0.15***	3.14 ± 0.34	5.94 ± 0.21*	6.81 ± 0.60**
	A. sativum	≤10	2.95 ± 0.21	5.29 ± 0.32**	6.58 ± 0.50**	8.79 ± 0.08**	4.59 ± 0.04*	5.11 ± 0.47**	6.40 ± 0.01**
	P. nigrum		2.98 ± 0.23	5.84 ± 0.32**	6.79 ± 0.08**	7.62 ± 1.00***	4.40 ± 0.01*	5.14 ± 0.21**	6.91 ± 0.21**
	M. myristica		2.98 ± 0.19	5.12 ± 0.16**	7.11 ± 0.3**	8.43 ± 0.20**	3.61 ± 0.23*	5.47 ± 0.11**	7.14 ± 0.21**
Peroxide value (mEqO$_2$/kg)	Control		3.57 ± 0.34	4.60 ± 0.04*	6.11 ± 0.80*	7.50 ± 0.20*	3.87 ± 0.20	4.95 ± 0.34*	5.83 ± 0.60*
	BHT		2.99 ± 0.37	3.94 ± 0.90*	5.68 ± 0.01*	6.29 ± 0.03**	3.69 ± 0.10	4.16 ± 0.22	5.22 ± 0.30*
	A. sativum	≤5	3.27 ± 0.22	4.30 ± 0.09*	5.28 ± 0.26*	6.69 ± 0.15**	4.12 ± 0.30	4.82 ± 0.20*	5.69 ± 0.27*
	P. nigrum		3.46 ± 0.16	4.51 ± 0.11*	5.20 ± 0.32*	6.72 ± 0.26**	3.77 ± 0.44	4.52 ± 0.12*	5.40 ± 0.16*
	M. myristica		3.40 ± 0.19	4.69 ± 0.02*	6.15 ± 0.03**	6.94 ± 0.80*	3.57 ± 0.25	4.63 ± 0.41*	5.11 ± 0.12*
Anisidine value	Control		3.32 ± 0.80	6.81 ± 0.59*	9.24 ± 0.51**	13.08 ± 1.11***	5.31 ± 0.27*	7.16 ± 0.89*	9.09 ± 1.27**
	BHT		3.19 ± 0.51	5.33 ± 0.97*	7.40 ± 1.75*	10.88 ± 2.11***	3.60 ± 0.88	5.08 ± 1.11*	7.05 ± 1.20*
	A. sativum	≤20	3.26 ± 0.70	6.03 ± 0.64*	8.44 ± 2.26*	10.49 ± 0.82***	4.27 ± 1.80	6.18 ± 0.97*	8.40 ± 2.27*
	P. nigrum		3.26 ± 1.13	6.93 ± 0.47*	9.39 ± 0.41**	11.08 ± 1.11***	4.06 ± 0.67	6.40 ± 2.27*	8.96 ± 1.05**
	M. myristica		3.29 ± 0.54	6.57 ± 0.39*	8.99 ± 0.93**	11.65 ± 2.06***	4.57 ± 2.70	6.18 ± 1.95*	8.77 ± 2.01**
TOTOX	Control		10.46 ± 0.57	16.01 ± 0.31**	21.46 ± 0.65***	28.08 ± 0.66***	13.05 ± 0.23*	15.73 ± 1.23**	20.75 ± 0.94***
	BHT		9.17 ± 0.44	13.21 ± 0.97*	18.76 ± 0.88***	23.46 ± 1.07***	10.74 ± 0.57	14.34 ± 0.76**	17.27 ± 0.66***
	A. sativum	—	9.80 ± 0.46	14.63 ± 0.37*	19.00 ± 1.26**	23.87 ± 0.49***	11.65 ± 0.95	14.50 ± 0.59**	18.84 ± 1.29***
	P. nigrum		10.18 ± 0.64	15.95 ± 0.29**	19.79 ± 0.36**	24.52 ± 0.69***	12.30 ± 0.49	16.04 ± 1.18**	20.34 ± 0.66***
	M. myristica		10.09 ± 0.37	15.95 ± 0.21**	19.29 ± 0.48**	25.53 ± 1.43**	12.11 ± 1.57	15.22 ± 1.04**	19.57 ± 1.09***

BTH: butylhydroxytoluene; *significantly different at $P < 0.05$ as compared to day 0; **significantly different at $P < 0.01$ as compared to day 0; ***significantly different at $P < 0.001$ as compared to day 0. In bold, we have values for oil freshly extracted.

TABLE 2: Effect of spices and temperature storage on the antibacterial activity of *Fontitrygon margarita* liver oil.

Bacteria		Treatment	ATB	CMI (mg/ml) FISH oil						
					Storage					
				Day 0	Room temperature (28 ± 2°C)			Refrigeration (4°C)		
Species	Strain codes				Day 60	Day 120	Day 180	Day 60	Day 120	Day 180
Escherichia coli	ATCC 10536	Control		**32**	64	128	256	32	64	128
		BHT		**32**	32	64	128	32	64	64
		A. sativum	16	**16**	32	64	128	32	32	64
		P. nigrum		**32**	64	128	256	32	64	64
		M. myristica		**32**	64	64	128	32	64	128
	EC 137	Control		**16**	32	64	128	16	32	64
		BHT		**16**	32	128	128	32	64	64
		A. sativum	8	**16**	32	64	64	32	64	64
		P. nigrum		**16**	32	64	256	32	64	64
		M. myristica		**32**	32	64	128	64	64	128
Enterobacter cloacae	ENT 119	Control		**16**	32	64	128	16	32	64
		BHT		**16**	32	64	64	32	64	64
		A. sativum	8	**16**	32	64	128	32	32	64
		P. nigrum		**16**	64	128	256	32	64	64
		M. myristica		**32**	32	64	64	64	128	128
	ENT 51	Control		**16**	32	64	128	16	32	64
		BHT		**16**	64	128	128	32	64	128
		A. sativum	8	**16**	32	64	128	32	32	64
		P. nigrum		**16**	32	64	128	32	64	64
		M. myristica		**16**	32	128	128	32	64	128
Klebsiella pneumonia	KL 11	Control		**16**	64	128	256	32	64	64
		BHT		**16**	32	64	128	32	64	64
		A. sativum	4	**16**	32	64	128	32	64	64
		P. nigrum		**32**	64	128	256	32	64	64
		M. myristica		**16**	32	64	128	32	64	64
Salmonella enterica serovar typhi	SAL 9	Control		**32**	64	128	256	64	64	128
		BHT		**16**	64	128	128	32	64	64
		A. sativum	4	**32**	64	128	128	32	32	64
		P. nigrum		**16**	64	128	128	32	64	128
		M. myristica		**32**	64	128	256	32	64	64
	ATCC 28579	Control		**16**	64	128	256	32	64	128
		BHT		**16**	64	128	128	32	128	128
		A. sativum	4	**16**	32	64	64	64	64	64
		P. nigrum		**16**	32	64	128	32	64	128
		M. myristica		**16**	32	64	128	32	128	128
Citrobacter freundii	CITB 81	Control		**32**	64	128	256	64	64	128
		BHT		**32**	64	128	128	64	64	128
		A. sativum	16	**32**	64	128	128	64	64	128
		P. nigrum		**32**	64	128	256	64	64	128
		M. myristica		**32**	64	128	128	64	64	128
Yersinia enterocolitica	YERB 1	Control		**32**	32	64	128	32	64	64
		BHT		**16**	64	128	256	32	64	128
		A. sativum	16	**16**	32	64	128	32	32	64
		P. nigrum		**16**	32	64	128	32	32	64
		M. myristica		**16**	64	128	128	32	64	128
Staphylococcus aureus	ST 120	Control		**32**	64	128	256	64	64	128
		BHT		**32**	32	64	128	64	64	128
		A. sativum	8	**16**	64	128	128	32	32	64
		P. nigrum		**32**	64	128	256	32	64	64
		M. myristica		**32**	64	128	256	32	64	128

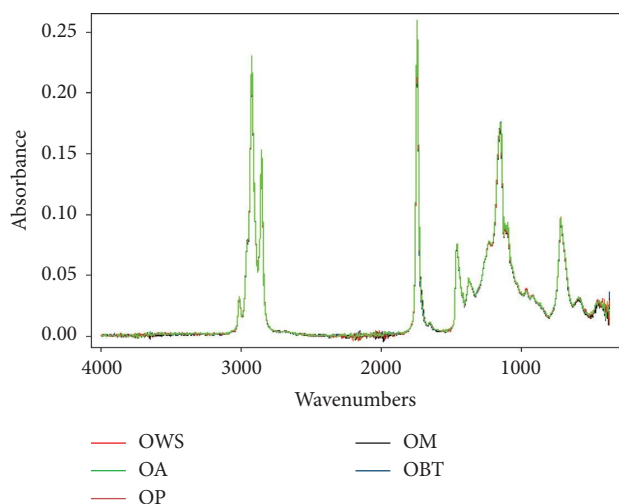BTH: butylhydroxytoluene. In bold, we have values for oil freshly extracted.

Figure 1: *Fontitrygon margarita* liver oil FITR profile according to stabilizers. OWS: oil extracted without spices; OA: oil extracted with *Allium sativum*; OP: oil extracted with *Piper nigrum*; OM: oil extracted with *Monodora myristica*; OBT: oil extracted with BHT.
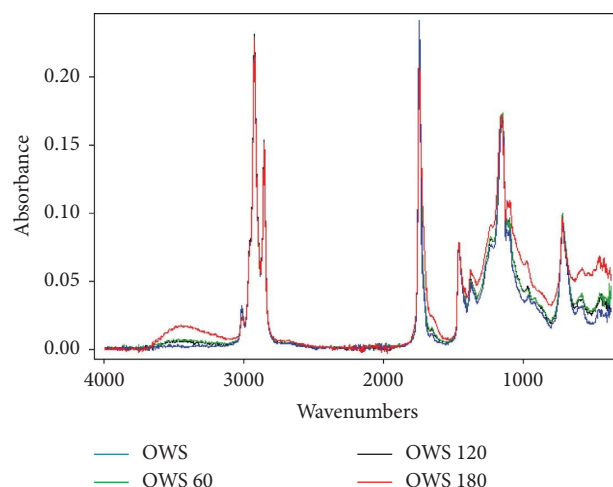

Figure 2: FTIR profile of *Fontitrygon margarita* liver oil according to room temperature storage time. OWS: oil extracted without spices at day 0; OWS 60, OWS 120, and OWS 180: oils extracted without spices stored at room temperature for 60, 120, and 180 days, respectively.

double bond and initiates the oxidation reaction. Almeck et al. [36] showed that the acid and peroxide index of *Canarium schweinfurthii* fruit pulp oil packaged in transparent bottles increased significantly during storage at $25°C$ (room temperature). The acid index increased from 6.93 mgKOH/g on the extraction day to 8.77 mgKOH/g after 6 months of storage, while the peroxide index went from 3.4 mEqO$_2$/kg to 17.06 mEqO$_2$/kg in the same time interval. In addition, autoxidation could be the cause of the increased formation of peroxides during storage.

Figure 2 presents the FTIR spectra of *F. margarita* liver oil extracted without stabilizers (control) and stored at room temperature over time. Unlike the spectra in Figure 1, those in Figure 2 show the presence of peaks at $3400 \, cm^{-1}$, indicating the presence of hydroxyl groups. This peak is more pronounced for oil samples stored for 180 days. This result shows that hydroperoxides are formed gradually during storage at room temperature. This could also suggest that long-term exposure of the oil at room temperature favors the hydrolysis of triglycerides, leading to the formation of free fatty acids. Similar bands were found when analyzing the lipid oxidation of catfish after cooking and smoking by different methods oil [37], and in oils from Lutjanus dentatus oil extracted by drying at $45°C$ for 24 hours or by cooking in a pressure cooker at $95°C$ for 20 minutes [38]. The peak at $3014 \, cm^{-1}$ characteristic of the cis double bond (=CH) provides information on the degree of lipid unsaturation. This peak was high at extraction and low after 180 days of storage. The decrease reflects the degradation of the double bonds during storage. This observation correlated with the iodine value result. The peak at $1654 \, cm^{-1}$ attributed to the vibration strain of the elongation of C=C (cis) and those appearing at $718 \, cm^{-1}$ linked to the vibration in the molecules analyzed of the double cis bonds also reflect the double bond present in unsaturated fatty acid oil. These peaks are observable in the sample before storage and are less

pronounced in the samples stored for 120 and 180 days at room temperature. The major structural modification of polyunsaturated lipids during oxidation reactions is the decrease in ethylenic double bonds caused by the attack of oxygen with the formation of oxidation compounds [39]. The band characteristic of the C=O ester group of triglycerides was found around $1745 \, cm^{-1}$, whereas the values of $1746 \, cm^{-1}$, $1743 \, cm^{-1}$, and $1750 \, cm^{-1}$ were reported by Guillèn et al. [40], Giménez et al. [41], and Tenyang et al. [37, 42], respectively. The sample stored for 180 days presented the weakest band. This means that this sample had low carbonyl groups of ester linkage of triglycerides and stipulated that alterations in this sample are higher. This is in agreement with the results obtained by Tenyang et al. [37, 42].

3.2.2. Antibacterial Properties. As noted in the quality indices, the antibacterial activity of *F. margarita* liver oil gradually decreases during storage at room temperature, as shown in Table 2. In fact, the MICs of the oil on bacterial strains of *E. coli* (EC 137), *E. cloacae* (ENT 119 and ENT 51), and *Y. enterocolitica* (YERB 1) increased from 16 mg/mL on day 0 to 128 mg/mL after 180 days of storage at room temperature. A similar reduction in antibacterial activity was observed on the bacterial strains of *E. coli* (ATCC 0536), *S. typhi* (SAL 9), *C. freundii* (CITB 81), and *S. aureus* (ST 120), which increased from 32 mg/mL at day 0 to 256 mg/mL at the 180th day of storage at room temperature. This could be due to the reduction in fatty acids present in the oil, whose antibacterial properties have been demonstrated by several researchers. Generally, unsaturated FFAs have greater antibacterial potential than saturated FFAs with the same carbon chain length. Also, a direct correlation exists between the number of double bonds in an unsaturated FFA's carbon chain and its antibacterial efficacy [43, 44].

3.3. Stabilization of F. margarita Liver Oil Properties

3.3.1. Effect of Storage Temperature

(1) Physicochemical Parameters. Figure 3 illustrates the AUC of the quality indices of *F. margarita* liver oil according to the storage temperature. It is observed that, regardless of the quality index, the oil stored in the refrigerator had a significantly lower AUC than the oil stored at room temperature, for both oxidation markers and acid value. This indicates that refrigeration slows down the deterioration of fish oil, likely because low temperatures limit the destruction of C=C double bonds. Haffaf and Lardjane [45] demonstrated that oil stored in the refrigerator slowed down the evolution of the peroxide index compared with oil exposed to the sun. Similarly, Almeck et al. [36] showed that storage of *Canarium schweinfurthii* fruit pulp oil in a refrigerator slowed the formation of hydroperoxides compared with oil stored at 25°C (room temperature). As shown in Table 1, at the 180th day of storage, the acid and thiobarbituric acid values were 3.72 ± 0.10 mg KOH/g and $8.16 \pm 0.27 \mu$mol MDA/Kg when stored in a refrigerator, compared with 5.32 ± 0.20 mg KOH/g and $10.54 \pm 0.30 \mu$mol MDA/Kg for oil stored at room temperature, respectively. Also, when stored at room temperature, the peroxide value (6.11 ± 0.80 meqO$_2$/kg) exceeded the recommended limit after 120 days of storage, while it remained within the normal range (4.95 ± 0.34 meqO$_2$/kg) after the same period when stored in the refrigerator.

(2) Antibacterial Properties. Figure 4 illustrates the AUC of the antibacterial activity of *F. margarita* liver oil, with a focus on the impact of storage temperature. In particular, oil stored in the refrigerator demonstrated a significantly lower AUC across all tested bacterial strains than oil stored at room temperature. This suggests that the antibacterial activity of *F. margarita* liver oil is better preserved in a refrigerator, potentially due to the refrigeration slowing down the degradation of the C=C double bonds in the oil's unsaturated fatty acids, which are believed to be responsible for its antibacterial properties. Herndon et al. [4] and Noutsa et al. [2] reported that the antibacterial activity of fish oil is positively correlated with unsaturation levels. As shown in Table 2, the MIC of oils ranged from 16 mg/mL to 128 mg/mL for the strains of *E. coli* (EC 137), *Y. enterocolitica* (YERB 1), *E. cloacae* (ENT 51), and *K. pneumoniae* (KL 11) at the 180th day when stored at room temperature. However, for oil stored in a refrigerator, the MIC only increased from 16 mg/mL to 64 mg/mL for these bacteria within the same period. Similarly, the MIC of oil increased from 32 mg/mL to 256 mg/mL for the strains of *E. coli* (ATCC 10536), *S. typhi* (SAL 9), *C. freundii* (CITB 81), and *S. aureus* (ST 120) at the 180th day of storage at room temperature, while the MICs only varied from 32 mg/mL to 128 mg/mL for these bacteria within the same period for oil stored in a refrigerator. In conclusion, storing *F. margarita* liver oil in a refrigerator appears to better maintain its antibacterial activity compared with storage at room temperature.

3.3.2. Effect of Stabilizers

(1) Physicochemical Parameters. Figure 5 demonstrates that *F. margarita* liver oils extracted with *A. sativum*, *P. nigrum*, *M. myristica*, and BHT had a significantly lower AUC than in the control oil (extracted without a stabilizer). This suggests that the inclusion of spices significantly mitigated the deterioration of fish oil stored at room temperature by decelerating the oxidation reaction. The antioxidant activity of these spices is often linked to the presence of phenolic compounds, which are known to capture free radicals and inhibit the release of superoxide radicals [46, 47]. This finding aligns with the work of Womeni et al. [27], who found that the rate of decrement in the iodine value of crude soybean oil was higher in the oil without spices than that containing the spices, *Z. officinale*, *X. parviflora*, *M. myristica*, *A. sativum*, and others. Similarly, Loungaing et al. [48] observed that the peroxide and anisidine index of palm oleic acid enriched with ginger extract had a significantly slower evolution during frying than in the oil sample not containing spices. In the comparative analysis of spices, *A. sativum* demonstrated the most significant efficacy, as evidenced by its lower AUC values relative to *P. nigrum* and *M. myristica* across various metrics, including acid, iodine, thiobarbituric acid, peroxide, and anisidine values. This potent activity correlates with the phenolic content found in these spices, with *A. sativum* extract showing higher levels than its counterparts. Consequently, *A. sativum* may offer superior effectiveness in maintaining fish oil quality throughout storage.

(2) Antibacterial Properties. As depicted in Figure 6, the AUC of the antibacterial activity of *F. margarita* liver oil stored at room temperature with the addition of spices shows interesting results. In particular, oil extracted with *A. sativum* and *M. myristica* had a significantly lower AUC than oils extracted without spices on 7 and 6 bacterial strains tested, respectively. In contrast, oil extracted with *P. nigrum* showed a significantly low AUC compared with the control oil extracted without spices on only 3 bacterial strains and was comparable on 4 other bacterial strains. This suggests that the addition of *A. sativum*, *M. myristica*, and *P. nigrum* helped to better preserve the antibacterial activity of *F. margarita* liver oil compared with when no spice was added. This could be due to the active principles contained in these spices as phenolic compounds, which, along with their antibacterial properties, may have acted as adjuvants by modulating the antibacterial activity of the oil. As shown in Table 2, after 180 days of storage at room temperature, the best antibacterial activity (CMI = 64 mg/mL) was obtained in oil extracted with *A. sativum* on *E. coli* (EC 137) and *S. typhi* (SAL 9; ATCC 28579). This was followed by oil extracted with *M. myristica*, which presented 7 CMIs of 128 mg/mL and 3 CMIs of 256 mg/mL. Cheradi and Sarni [49] demonstrated that bacterial strains of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *Escherichia coli* showed sensitivity to the aqueous extract of garlic. In addition to phenolic compounds, sulfur compounds such as allicin and diallyl disulfide present in garlic are believed to be responsible for
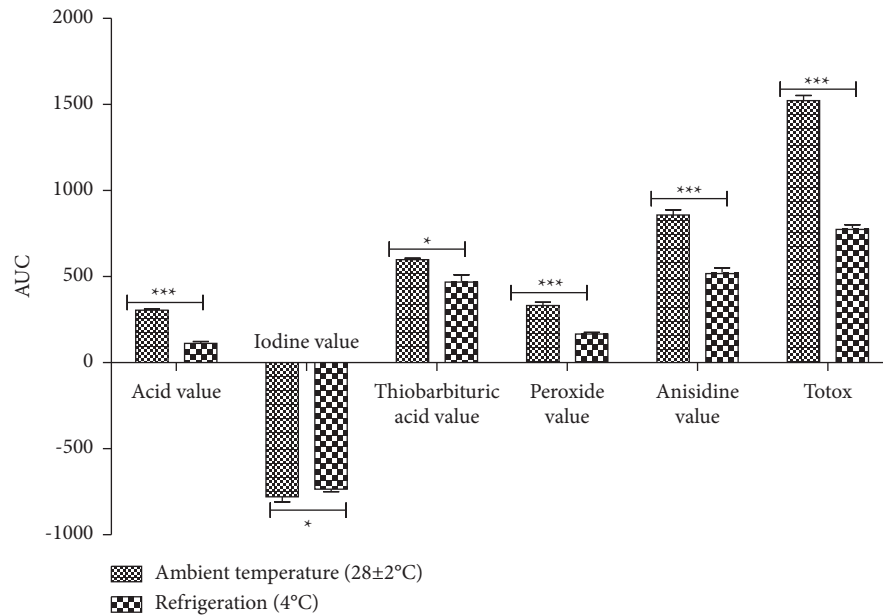
FIGURE 3: AUC of the quality indices of *Fontitrygon margarita* liver oil according to the storage temperature. *significant difference at $P < 0.05$; ***significant difference at $P < 0.001$.
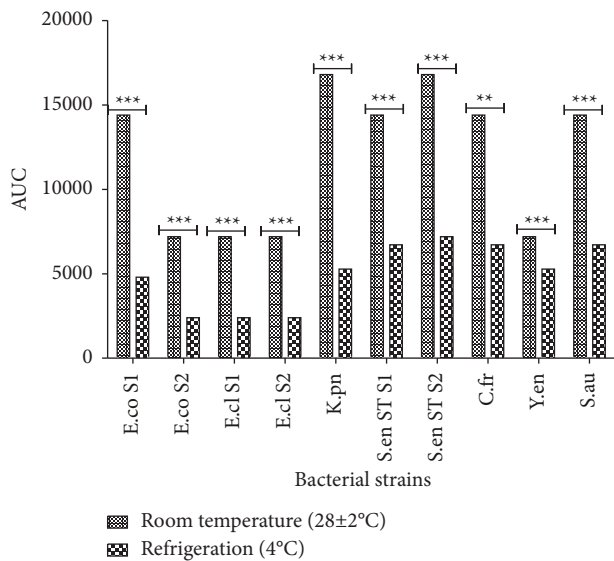

FIGURE 4: AUC of the antibacterial activity of *Fontitrygon margarita* liver oil as a function of the storage temperature. **significant difference at $P < 0.01$; ***significant difference at $P < 0.001$.

this antibacterial activity. Oil extracted with *P. nigrum*, as well as the control sample without spices, each had 4 MICs of 128 mg/mL and 6 MICs of 256 mg/mL on the bacterial strains. Moreover, the addition of *A. sativum* and *M. myristica* overall better preserved the antibacterial activity of *F. margarita* liver oil during storage, especially on bacterial strains of *E. coli* (EC 137), *E. cloacae* (ENT 51 and ENT 119), *S. typhi* (SAL 9; ATCC 28579), and *Y. enterocolitica* (YERB 1) compared with the oil sample extracted with BHT.

3.3.3. Combined Effect of Storage Temperature and Stabilizers.

Figure 7 provides an overview of the AUC of the oil quality indices, taking into account both storage temperature and the addition of spices. Notably, for the thiobarbituric acid index, anisidine values, and total oxidation, the oils extracted with *A. sativum*, *P. nigrum*, *M. myristica*, and BHT and stored in a refrigerator had significantly lower AUC than oils extracted with spices and stored at room temperature, and those extracted without spices and stored in a refrigerator. Furthermore, the iodine value of these oils had a significantly low AUC compared with oil extracted with spices stored at room temperature and was lower or equal to oils extracted without spices stored in a refrigerator. This suggests that the combination of adding spices to *F. margarita* liver oil and storing it in a refrigerator significantly reduced the deterioration process of the oil compared with just adding spices or only storing it in a refrigerator. As previously mentioned, both refrigeration temperature and the addition of spices are limiting factors for oil oxidation [27, 45]. Regarding acid and peroxide values, with the exception of *A. sativum* spice, oils extracted with stabilizers had a significantly lower AUC than oil extracted with spices stored at room temperature and were lower or equal to oil extracted without spices and stored in a refrigerator.

(1) Antibacterial Properties. Figure 8 provides a comparison of the AUC of the antibacterial activity of *F. margarita* liver oil, considering both storage temperature and the addition of spices. It is evident that oils extracted with various spices (*A. sativum*, *P. nigrum*, and *M. myristica*) and stored in a refrigerator had a significantly lower AUC than oils extracted with spices but stored at room temperature across all tested bacterial strains. The only exception was the oil extracted with *M. myristica* from the bacterial strains of
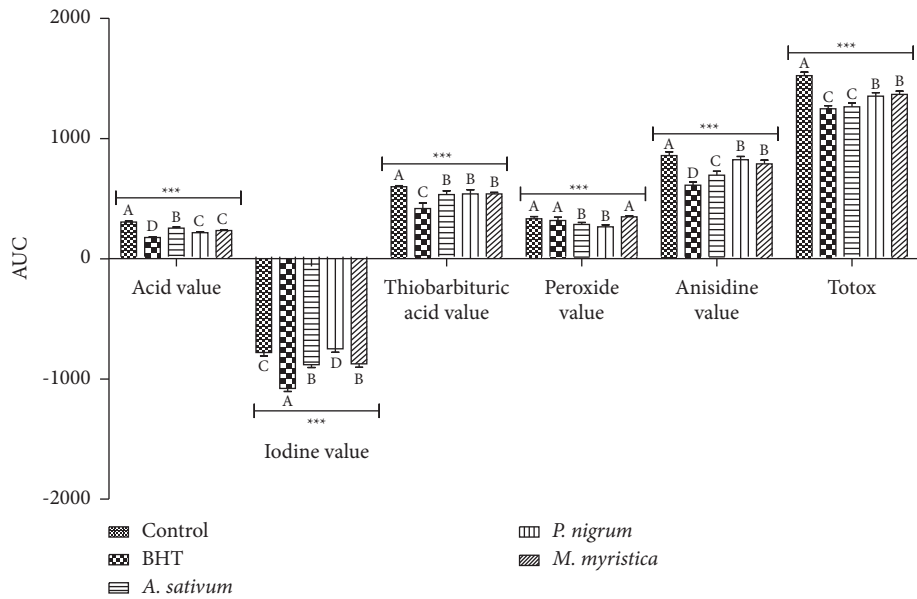
FIGURE 5: AUC of the quality indices of *Fontitrygon margarita* liver oil as a function of the spices. For a given parameter, values with the same letter are not significantly different. ***significant difference at *P* < 0.001.
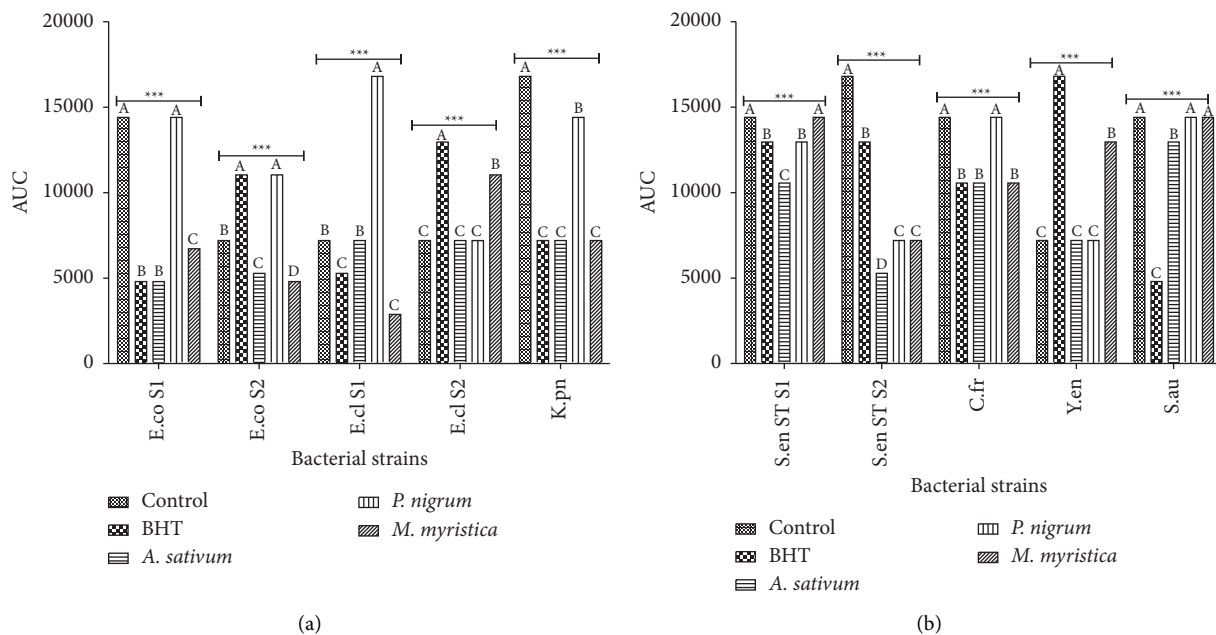

(a)

(b)

FIGURE 6: AUC of the antibacterial activity of *Fontitrygon margarita* liver oil according to the spices. (a) corresponds to the strains *Escherichia coli* (strain ATCC 10536), *Escherichia coli* (strain EC 137), *Enterobacter cloacae* (strain ENT 119), *Enterobacter cloacae* (strain ENT 51) and *Klebsiella pneumonia* (strain KL 11); and (b) to the strains *Salmonella enterica serovar typhi* (strain SAL 9), *Salmonella enterica serovar typhi* (strain ATCC 28579), *Citrobacter freundii* (strain CITB 81), *Yersinia enterocolitica* (strain YERB 1), and *Staphylococcus aureus* (strain ST 120). For a given bacterial strain, values with the same letter are not significantly different. ***significant difference at *P* < 0.001.

E. coli (EC 137), *E. cloacae* (ENT 119), and *S. typhi* (ATCC 28579). This reinforces the observation that storage in a refrigerator helps preserve the antibacterial activity of the oil compared with storage at room temperature. Moreover, the oil extracted with these spices and stored in a refrigerator had an AUC less than or equal to oil samples extracted without spices but stored in a refrigerator, particularly on the bacterial strains of *E. coli* (ATCC 10536), *K. pneumoniae* (KL

11), *S. typhi* (ATCC 28579; SAL 9), *S. aureus* (ST 120), *Y. enterocolitica* (YERB 1), and *C. freundii* (CITB 81). The only exception was the oil extracted with *M. myristica* from the bacterial strains of *S. typhi* (ATCC 28579) and *Y. enterocolitica* (YERB 1). This indicates that the combination of spices and storage in a refrigerator incrementally sustained the antibacterial activity of the oil compared with just storing it in a refrigerator.
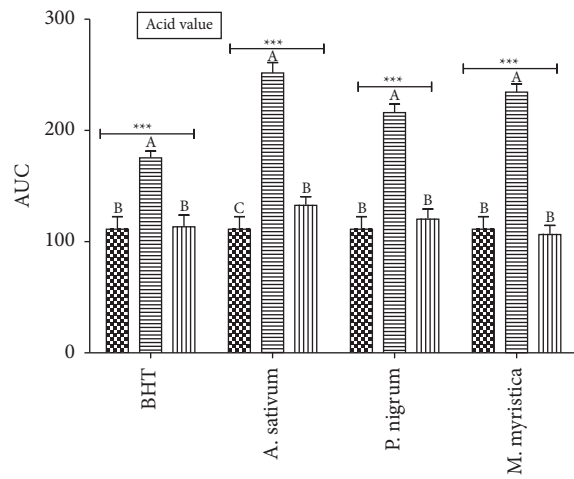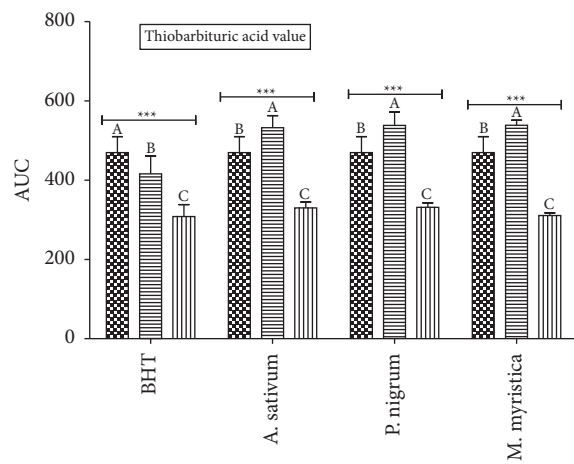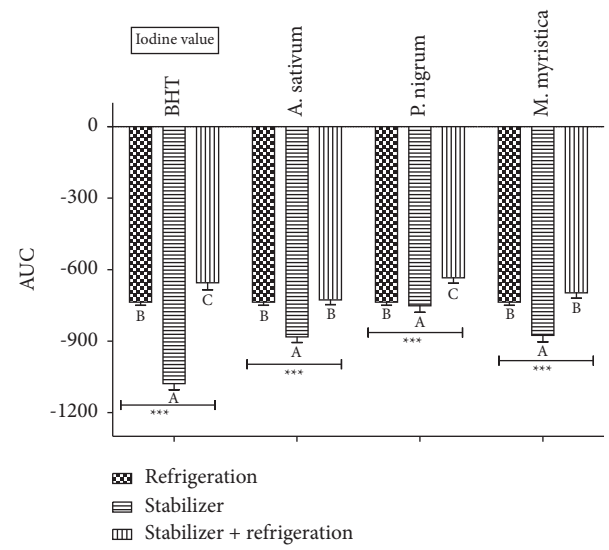
(a)

(b)

(c)
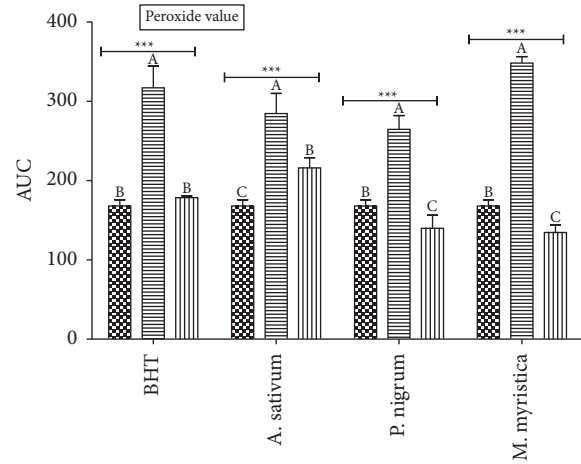
(d)

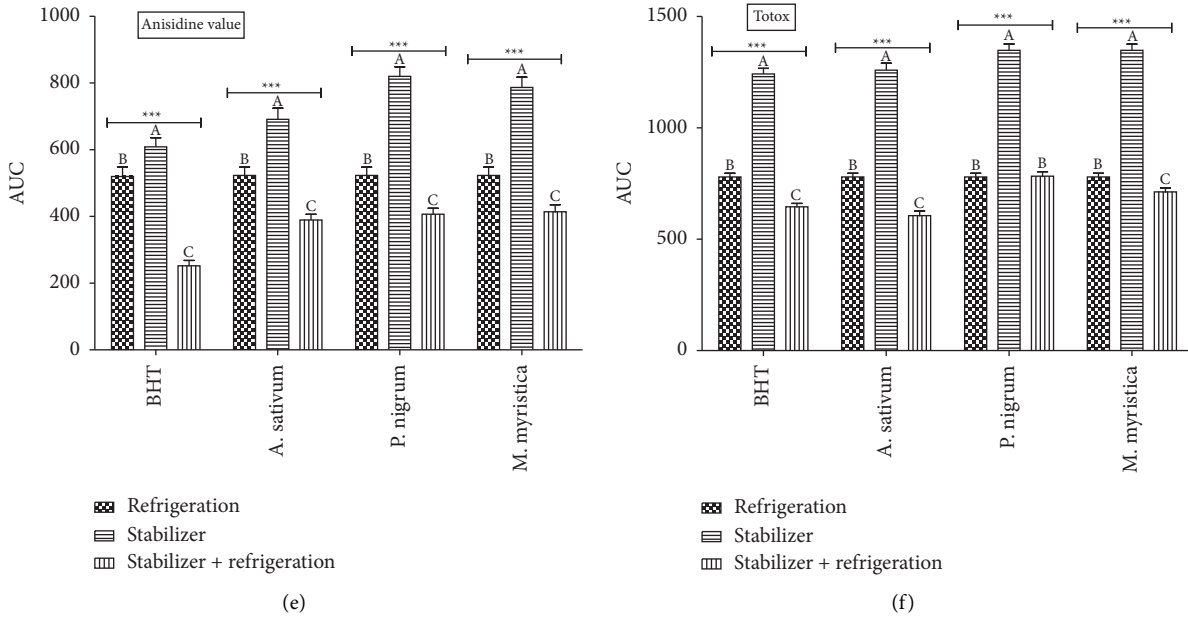Figure 7: Continued.

(e)

(f)

Figure 7: AUC of the quality indices of *Fontitrygon margarita* liver oil as a function of storage temperature and addition of spices. (a) Acid value, (b) Iodine value, (c) thiobarbituric acid value, (d) peroxide value, (e) anisidine value, (f) TOTOX. For a given parameter and for each stabilizer, values with the same letter are not significantly different. ***significant difference at $P < 0.001$.
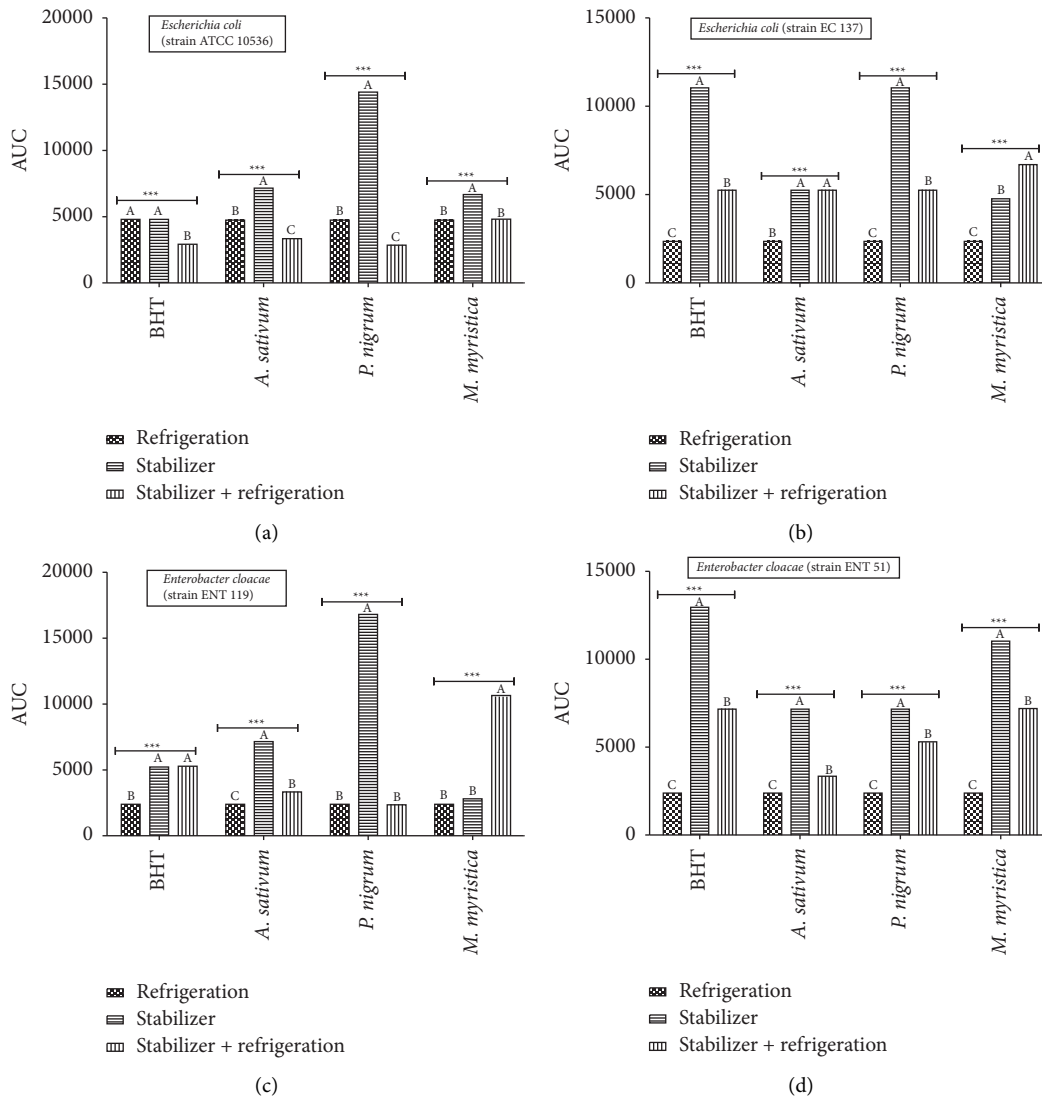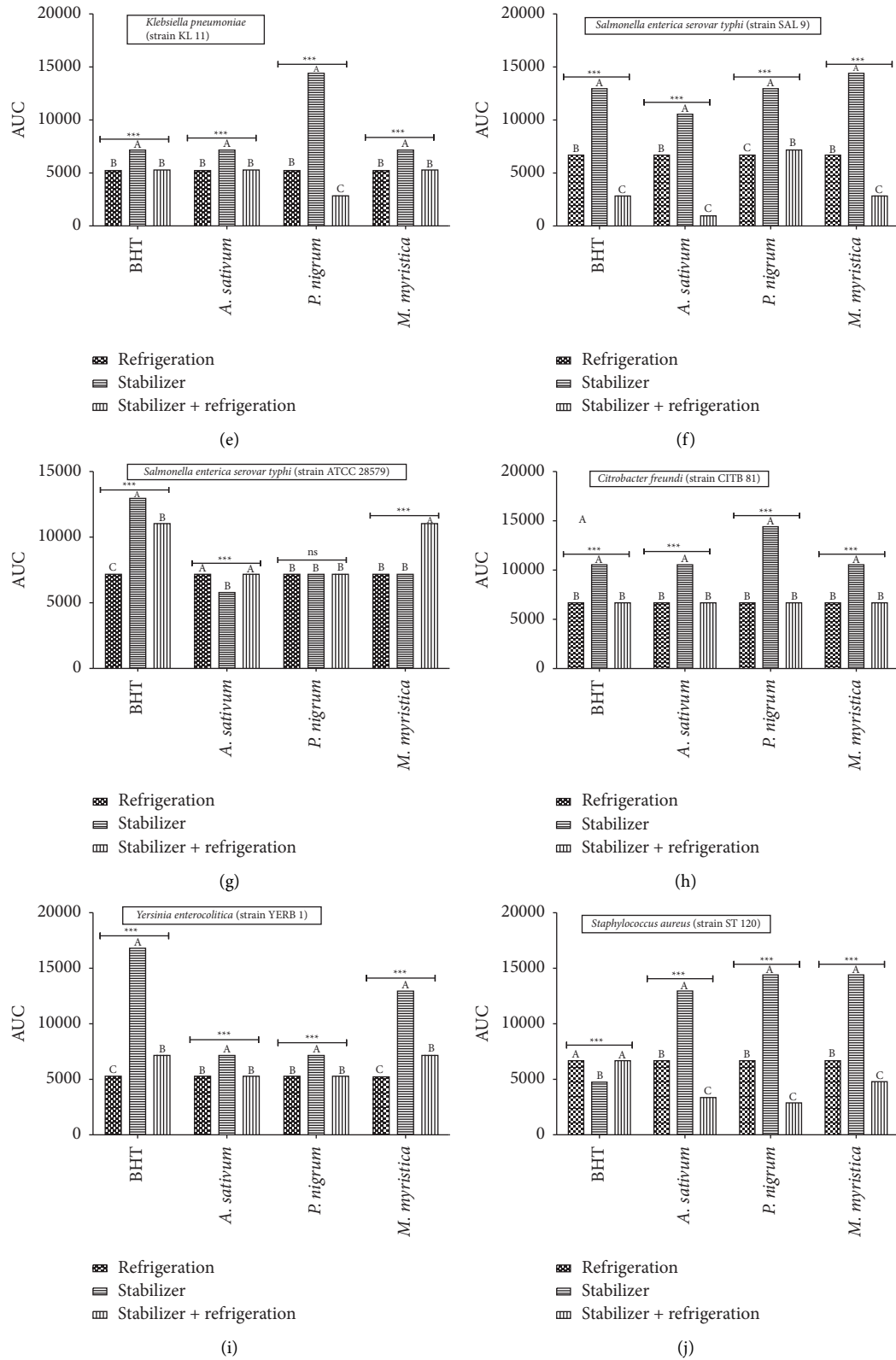

(a)

(b)

(c)

(d)

Figure 8: Continued.

FIGURE 8: AUC of the antibacterial activity of *Fontitrygon margarita* liver oil as a function of the storage temperature and the addition of spices. (a) *Escherichia coli* (strain ATCC 10536), (b) *Escherichia coli* (strain EC 137), (c) *Escherichia coli* (strain ENT 119), (d) *Escherichia coli* (strain ENT 51), (e) *Escherichia coli* (strain KL 11), (f) *Salmonella enterica serovar typhi* (strain SAL 9), (g) *Salmonella enterica serovar typhi* (strain ATCC 28579), (h) *Citrobacter freundii* (strain CITB 81), (i) *Yersinia enterocolitica* (strain YERB 1), (j) *Staphylococcus aureus* (strain ST 120). For a given bacterial strain and for each stabilizer, values with the same letter are not significantly different. ***significant difference at *P* < 0.001.

4. Conclusion

The study of *Fontitrygon margarita* liver oil has revealed that both storage duration and temperature significantly impact the oil's quality and antibacterial properties. Over time, the quality index and antibacterial activities of the oil tend to decrease. The degradation of oil quality is more pronounced when the oil is stored at room temperature. However, the addition of stabilizers to *F. margarita* liver oil prior to extraction, or storing the oil in a refrigerator, can mitigate this degradation by preserving the oil's chemical composition and antibacterial properties. The combination of adding stabilizers and storing *F. margarita* liver oil in a refrigerator proved to be the most effective method for preserving the oil's quality and antibacterial properties during storage. Given its antibacterial properties, *F. margarita* liver oil holds significant potential for the nutraceutical industry and could be used as a dietary supplement. This research underscores the importance of proper storage conditions and the use of stabilizers in maintaining the quality of such valuable natural resources.

Looking ahead, future research should focus on the oil's long-term stability, examining its shelf life over extended periods and the impact of various stabilizing agents. Studies should also delve into the influence of natural stabilizer concentrations and quantify the levels of phenolic compounds present in the oils. To establish the oil's suitability for health-related uses, clinical trials are essential to evaluate its effectiveness and safety profile as a dietary supplement or in other therapeutic contexts. Exploring alternative extraction techniques could potentially enhance the oil's quality or yield without compromising its intrinsic properties. Investigating the synergistic effects of combining *F. margarita* liver oil extracted with other natural substances could further amplify its antibacterial capabilities. A thorough investigation into the molecular mechanisms underlying the oil's antibacterial action could pave the way for targeted medical applications. Additionally, conducting a market analysis would shed light on potential commercial prospects and consumer preferences for products derived from *F. margarita* liver oil.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

References

[1] J. C. K. Manz, J. V. F. Nsoga, J. B. Diazenza et al., "Nutritional composition, heavy metal contents and lipid quality of five marine fish species from Cameroon coast," *Heliyon*, vol. 9, no. 3, Article ID e14031, 2023.

[2] B. S. Noutsa, S. R. Tchabong, A. D. D. Djitieu et al., "Chemical characterization and antibacterial properties of *Fontitrygon margarita* (Günther, 1870) liver oil," *Journal of Lipids*, vol. 2022, Article ID 9369387, 10 pages, 2022.

[3] J. Pinela, B. Rodrigues, M. Pires et al., "Upcycling fish by-products into bioactive fish oil: the suitability of microwave-assisted extraction," *Biomolecules*, vol. 13, no. 1, p. 18, 2023.

[4] J. L. Herndon, R. E. Peters, R. N. Hofer, T. B. Simmons, S. J. Symes, and D. K. Giles, "Exogenous polyunsaturated fatty acids (PUFAs) promote changes in growth, phospholipid composition, membrane permeability and virulence phenotypes in *Escherichia coli*," *BMC Microbiology*, vol. 20, pp. 305–312, 2020.

[5] A. M. Shah, W. Yang, H. Mohamed, Y. Zhang, and Y. Song, "Microbes: a hidden treasure of polyunsaturated fatty acids," *Frontiers in Nutrition*, vol. 9, Article ID 827837, 2022.

[6] P. Guo, L. Dong, F. Wang, L. Chen, and W. Zhang, "Deciphering and engineering the polyunsaturated fatty acid synthase pathway from eukaryotic microorganisms," *Frontiers in Bioengineering and Biotechnology*, vol. 10, Article ID 1052785, 2022.

[7] V. I. Lakshimi and M. Kavitha, "New insights into prospective health potential of ω-3 PUFAs," *Current Nutrition Reports*, vol. 12, no. 4, pp. 813–829, 2023.

[8] D. F. F. Dongho, M. A. R. Fogang, K. J. C. Manz et al., "Physicochemical characteristics and nutritional and biological properties of fish oils in Cameroon: an overview," *Journal of Food Quality*, vol. 2023, Article ID 7847288, 23 pages, 2023.

[9] R. Zokou, F. F. Dongho Dongmo, S. C. Ndomou Houketchang et al., "Influence of cooking methods on *Oreochromis niloticus* (Tilapia) oils antibacterial activity," *Food and Humanity*, vol. 1, pp. 921–927, 2023.

[10] G. Á. Quintero-Martínez, C. Hernández, E. Palacios, M. C. Chávez-Sánchez, L. Ibarra-Castro, and M. Á. Hurtado-Oliva, "Oxidized fish oil in the diet negatively affect rearing performance, health, and tissue fatty acid composition of juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869)," *Aquaculture International*, vol. 31, no. 6, pp. 3489–3511, 2023.

[11] W. Y. Oh, M. J. Kim, and J. Lee, "Approaches of lipid oxidation mechanisms in oil matrices using association colloids and analysis methods for the lipid oxidation," *Food Science and Biotechnology*, vol. 32, no. 13, pp. 1805–1819, 2023.

[12] M. D. Suárez-Medina, M. I. Sáez-Casado, T. Martínez-Moya, and M. Á. Rincón-Cervera, "The effect of low temperature storage on the lipid quality of fish, either alone or combined with alternative preservation technologies," *Foods*, vol. 13, no. 7, p. 1097, 2024.

[13] H. Lu, "Stabilization of fish oil with naturally sourced antioxidants," 2023.

[14] M. Kazuo, "Prevention of fish oil oxidation," *Journal of Oleo Science*, vol. 68, no. 1, pp. 1–11, 2019.

[15] V. Šimat, T. Bogdanović, V. Poljak, and S. Petričević, "Changes in fatty acid composition, atherogenic and thrombogenic health lipid indices and lipid stability of bogue (*Boops boops* Linnaeus, 1758) during storage on ice: Effect of fish farming activities," *Journal of Food Composition and Analysis*, vol. 40, pp. 120–125, 2015.

[16] G. Wu, C. Chang, C. Hong et al., "Phenolic compounds as stabilizers of oils and antioxidative mechanisms under frying conditions: a comprehensive review," *Trends in Food Science and Technology*, vol. 92, pp. 33–45, 2019.

[17] G. Kerr, "Do fish oil softgels expire?" 2019, https://www.livestrong.com/article/519288-do-fish-oil-softgels-expire/.

[18] A. Benedict, "Does fish oil expire? (Figure out whether it's gone bad)," 2024, https://totalshape.com/supplements/does-fish-oil-expire/.

[19] J. G. Tamba, F. E. Sapnken, T. W. E. Azong, S. Guefano, A. F. Lélé, and L. Monkam, "An overview of electricity in Cameroon: current status, influential factors and government actions," *International Journal of Energy Economics and Policy*, vol. 12, no. 4, pp. 470–481, 2022.

[20] J. Rani, P. Kaur, and C. Chuwa, "Nutritional benefits of herbs and spices to the human beings," *Annals of Phytomedicine An International Journal*, vol. 12, no. 1, pp. 187–197, 2023.

[21] Z. G. Tabanty, N. Tenyang, L. Birault et al., "Effect of some local plant extracts on fatty acid composition of fish (*Alestesbaremoze*) during smoking and sun drying in the Far North region of Cameroon," *Food Science and Nutrition*, pp. 1–17, 2023.

[22] S. Djiazet, L. B. Mezajoug Kenfack, E. Serge Ngangoum, H. Ghomdim Nzali, and C. Tchiégang, "Indigenous spices consumed in the food habits of the populations living in some countries of Sub-Saharan Africa: utilisation value, nutritional and health potentials for the development of functional foods and drugs: a review," *Food Research International*, vol. 157, Article ID 111280, 2022.

[23] T. C. L. Maguipa, P. D. Mbougueng, and H. M. Womeni, "Evaluation of the sensory attributes of pepper soup beef hides and determination of the preservative potential of the spices used for its preparation," *Journal of Agriculture and Food Research*, vol. 8, Article ID 100293, 2022.

[24] L. A. de la Rosa, N. M. R. del Rocío, J. A. Domínguez-Avila, and E. Alvarez-Parrilla, "Phenolic compounds in herbs and spices," in *Phenolic Compounds in Food: Characterization and Analysis*, pp. 333–353, CRC Press, Boca Raton, FL, USA, 2018.

[25] N. Singh and S. S. Yadav, "A review on health benefits of phenolics derived from dietary spices," *Current Research in Food Science*, vol. 5, pp. 1508–1523, 2022.

[26] A. Rauf, T. Abu-Izneid, M. Thiruvengadam et al., "Garlic (Allium sativum L.): its chemistry, nutritional composition, toxicity, and anticancer properties," *Current Topics in Medicinal Chemistry*, vol. 22, no. 11, pp. 957–972, 2022.

[27] H. M. Womeni, D. F. Tonfack, B. Tientcheu, and M. Linder, "Antioxidant potential of methanolic extracts and powder of some Cameroonian spices during accelerated storage of soybean oil," *Advances in Biological Chemistry*, vol. 3, no. 3, p. 10, 2013.

[28] O. U. Shirazi, M. M. A. K. Khattak, and N. A. M. Shukri, "Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices," *Journal of Pharmacognosy and Phytochemistry*, vol. 3, no. 3, pp. 104–108, 2014.

[29] J. R. Bonilla and J. L. Hoyos-Concha, "Métodos de extracción, refinación y concentración de aceite de pescado como fuente de ácidos grasos omega-3," *Ciencia y Tecnología Agropecuaria*, vol. 19, no. 3, pp. 645–668, 2018.

[30] American Oil Chemists' Society, *Official Methods and Recommended Practices of the AOCS*, American Oil Chemists' Society, Urbana, IL, USA, 1998.

[31] C. Paquot, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, Elsevier, Amsterdam, Netherlands, 2013.

[32] N. Zhang, Y. Li, S. Wen et al., "Analytical methods for determining the peroxide value of edible oils: a mini-review," *Food Chemistry*, vol. 358, Article ID 129834, 2021.

[33] Clsi, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2018.

[34] J. A. Eloff, "Sensitive and quick micro plaque method to determine the minimal inhibitory concentration of plant extracts for bacteria," *Planta Medica*, vol. 63, pp. 711–713, 1998.

[35] Codex Alimentarius Commission, *Standards for Fish Oils, CXS 329-217: Adopted in 2017, Amended in 2021*, FAO/WHO Food Standards Program, Rome, Italy, 2021.

[36] K. Almeck, D. Aboubakar, C. Tchiegang, and M. Parmentier, "Evolution of certain physico–chemical quality parameters of *Canarium schweinfurthii* Engl. fruit pulp oil during storage," *International Journal of Brain and Cognitive Sciences*, vol. 2, no. 3, pp. 249–257, 2008.

[37] N. Tenyang, H. M. Womeni, B. Tiencheu et al., "Lipid oxidation of catfish (*Arius maculatus*) after cooking and smoking by different methods applied in Cameroon," *Food and Nutrition Sciences*, vol. 4, no. 9, pp. 176–187, 2013.

[38] N. B. Simo, D. A. D. Deutchoua, R. Zokou et al., "Chemical quality and antibacterial activity of *Lutjanus dentatus* (dumeril, 1860) oils as a function of extraction method," *Research Square Preprint*, 2020.

[39] A. A. Dzhatdoeva, A. M. Polimova, E. V. Proskurnina, M. A. Proskurnin, and Y. A. Vladimirov, "Determination of lipids and their oxidation products by IR spectrometry," *Journal of Analytical Chemistry*, vol. 71, no. 6, pp. 542–548, 2016.

[40] M. D. Guillén, A. Ruiz, and N. Cabo, "Study of the oxidative degradation of farmed salmon lipids by means of Fourier transform infrared spectroscopy. Influence of salting," *Journal of the Science of Food and Agriculture*, vol. 84, no. 12, pp. 1528–1534, 2004.

[41] B. Giménez, M. Gómez-Guillén, M. Pérez-Mateos, P. Montero, and G. Márquez-Ruiz, "Evaluation of lipid oxidation in horse mackerel patties covered with borage containing film during frozen storage," *Food Chemistry*, vol. 124, no. 4, pp. 1393–1403, 2011.

[42] N. Tenyang, B. Tiencheu, F. Tonfack Djikeng, A. T. Morfor, and H. M. Womeni, "Alteration of the lipid of red carp (*Cyprinus carpio*) during frozen storage," *Food Science and Nutrition*, vol. 7, no. 4, pp. 1371–1378, 2019.

[43] B. K. Yoon, J. A. Jackman, E. R. Valle-González, and N. J. Cho, "Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications," *International Journal of Molecular Sciences*, vol. 19, no. 4, p. 1114, 2018.

[44] C. Borreby, E. M. S. Lillebæk, and B. H. Kallipolitis, "Anti-infective activities of long-chain fatty acids against foodborne pathogens," *FEMS Microbiology Reviews*, vol. 47, no. 4, 2023.

[45] S. Haffaf and T. Lardjane, *Effect of storage conditions on the physico-chemical characteristics of "Fleurial" oil. Master's*

thesis in agronomic sciences, vol. 74, Mouloud Mammeri University of Tizi-Ouzou. Algeria, Tizi-Ouzou. Algeria, 2018.

[46] F. T. Djikeng, H. M. Womeni, E. Anjaneyulu, M. S. L. Karuna, R. B. N. Prasad, and M. Linder, "Effects of natural antioxidants extracted from Cameroonian ginger roots on the oxidative stability of refined palm olein," *European Food Research and Technology*, vol. 244, no. 6, pp. 1015–1025, 2017.

[47] M. I. Generalić, D. Skroza, I. Ljubenkov, V. Šimat, S. Smole Možina, and V. Katalinić, "*In-vitro* antioxidant and antibacterial activity of Lamiaceae phenolic extracts: a correlation study," *Food Technology and Biotechnology*, vol. 52, no. 1, pp. 119–127, 2014.

[48] V. D. Loungaing, D. F. Tonfack, B. G. Teboukeu, N. H. F. Njike, G. T. Kamsu, and H. M. Womeni, "Effect of ginger extracts on palm olein quality during frying and impact of fried oils on some biological parameters of albino *wistar* rats," *Journal of Food Research*, vol. 11, no. 3, p. 14, 2022.

[49] D. Cheradi and C. Sarni, "Evaluation of the antibacterial activity of the aqueous extract of garlic (*Allium sativum)* and its application for the preservation of fresh Turkey meat," *Master Thesis in Agronomic Sciences*, Mouloud Mammeri University of Tizi-Ouzou. Algeria, Tizi-Ouzou. Algeria, 2016.