

Physicochemical and spectroscopic characterization, and evaluation of the antioxidant and anti-inflammatory potential of oils extracted from *Thunnus thynnus* and *Sardina pilchardus* coproducts

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SUMMARY: This work aims to valorize the coproducts of two pelagic species, bluefin tuna (*Thunnus thynnus*) and sardines (*Sardina pilchardus*), by the extraction and characterization of the oil coproducts through physicochemical and FTIR-ATR analysis and the in vitro evaluation of their antioxidant and anti-inflammatory potential. Determining the physicochemical parameters revealed that both oils tested are of good quality. The UV-visible absorption spectra presented a strong absorbance band at 310 nm. Therefore, oil from *S. pilchardus* and *T. thynnus* may predominate ω -3 and 9 in its spectrum. The FTIR-ATR spectrum showed bands and peaks corresponding to functional groups of the substances tested. Evaluation of the antioxidant and anti-inflammatory potential showed that the oil from *S. pilchardus* coproducts showed the highest reduction percentage and even the highest anti-inflammatory potential compared to *T. thynnus*. Coproduct oil offers significant therapeutic value as a substitute substance or therapeutic support for reducing oxidative stress and inflammation.

KEY-WORDS: Coproducts; FTIR-ATR; Oil; *Sardina pilchardus*; *Thunnus thynnus*.

RESUMEN: Caracterización mediante FTIR-ATR y evaluación del potencial antioxidante y antiinflamatorio del aceite extraído de coproductos de *Thunnus thynnus* y *Sardina pilchardus*. Este trabajo tiene como objetivo poner en valor los coproductos de dos especies pelágicas, atún rojo (*Thunnus thynnus*) y sardinas (*Sardina pilchardus*), mediante la extracción y caracterización de los coproductos de los aceites mediante análisis fisicoquímicos y FTIR-ATR y la evaluación in vitro de su potencial antioxidante y antiinflamatorio. La determinación de los parámetros fisicoquímicos mostró que ambos aceites ensayados son de buena calidad. Los espectros de absorción UV-visible presentaron una fuerte banda de absorbancia a 310 nm, por lo que, en los aceites de *S. pilchardus* y *T. thynnus* predominan ω -3 y 9 en su espectro. El espectro FTIR-ATR mostró bandas y picos correspondientes a grupos funcionales de las sustancias ensayadas. La evaluación del potencial antioxidante y antiinflamatorio mostró que el aceite de coproductos de *S. pilchardus* tiene el mayor porcentaje de reducción e incluso el mayor potencial antiinflamatorio en comparación con *T. thynnus*. El aceite de coproducto ofrece un valor terapéutico significativo como sustancia sustitutiva o apoyo terapéutico para reducir el estrés oxidativo y la inflamación.

PALABRAS CLAVE: Aceite; Coproductos; FTIR-ATR; *Sardina pilchardus*; *Thunnus thynnus*.

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1. INTRODUCTION

The amount of waste and coproducts produced by fish transformation is believed to be 50% of the weight of the fish. Without proper treatment, these pollutants are frequently dumped into the environment directly, leading to serious environmental issues (Balasa *et al.*, 2020). The coproducts contain potentially valuable ingredients (antioxidants, anti-stress, antihypertensives, collagens, pigments, etc.) and are primarily made up of heads, viscera, skins, bones, tails, fins, and filleting residues (Coppola *et al.*, 2021).

One potential solution to this problem is to implement a waste management strategy that incorporates converting these leftovers into products that may be sold. Studies have indicated that consistent consumption of supplements derived from fish can promote the synthesis of substances with a variety of health benefits, such as antihypertensive, antihyperlipidemic, and immunomodulatory effects (Mutalipassi *et al.*, 2021). Coproducts and their metabolites are at the heart of global efforts to discover novel physiologically active substances that produce secondary metabolites and macromolecules with anti-inflammatory and antioxidant properties (El-shafei *et al.*, 2021).

Polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are abundant in fish oil. These fatty acids can be utilized as a component of functional meals in the food industry or as a nutraceutical element in the pharmaceutical sector due to their numerous health benefits and necessity (Strobel *et al.*, 2020).

The purpose of the study is to value the coproducts of two pelagic species: sardines (*Sardina pilchardus*) and bluefin tuna (*Thunnus thynnus*). Thus, the goal is predicated based on the extraction, physicochemical, and FTIR-ATR characterization of the oil coproducts as well as the in vitro assessment of their anti-inflammatory and antioxidant potential.

2. MATERIALS AND METHODS

2.1. Samples

The experimental study was carried out on coproducts from two species of pelagic fish: sardines (*Sardina pilchardus*) and tuna (*Thunnus thynnus*). Sampling was carried out during February 2024 in the region of Salamandre (Mostaganem, Algeria) (35° 56' 00" north, 0° 05' 00" east) for the sardine species and in the region of Beni Saf (Ain Temouchent, Algeria) (35° 18' 8" North, 1° 23' 1" West) for the tuna species.

2.2. Extraction of oils from coproducts

The extraction was carried out according to the protocol described by Rubio-Rodriguez *et al.* (2010). Homogenized solid waste was added to water at a ratio of 1:1. The sample was heated to 75 °C for 15 minutes. After boiling, the homogenized sample was pressed to obtain the liquid fraction and stored in the refrigerator until centrifugation. Centrifugation was performed at 11180 g in 15 min to separate the water from the oil. Petroleum ether was then used to separate the oil from the water thoroughly. Then, the oil fraction was stored at 4 °C after the determination of extraction yield.

2.3. Physicochemical analysis of the oil from coproducts

Determinations of the physicochemical indexes (acidity, peroxide, saponification, iodine, and relative density) to characterize the oil from the coproducts were made according to Nascimento *et al.* (2015).

2.4. UV-Vis spectrophotometric analysis of oil

The analysis was conducted on a SPECORD® 210 PLUS UV/Visible spectrophotometer (Analytik Jena GmbH, Jena, Germany), which is a double-beam spectrophotometer designed for precise quantitative and qualitative analysis in the ultraviolet and visible spectral range (190–1200 nm). It features a Tempered Detector System that enhances measurement stability and accuracy. The instrument is operated using

WinASPECT® UV basic software, which allows for comprehensive spectral acquisition, data processing, and evaluation. 1 ml of the tuna or sardine coproduct oil was put into the spectrophotometer tank. Then, the absorbance and λ_{max} were determined.

2.5. Spectroscopic analysis (FTIR-ATR)

The analysis was carried out on a BRUKER ALPHA spectrometer equipped with an ATR stage with a diamond crystal triglycine sulfate detector and controlled by the Opus v 7.0 122 software. 1 g of the oil was directly deposited onto the reflection crystal of the Platinum-ATR accessory. The acquisitions were performed by taking 200 scans between 4000 and 500 cm^{-1} with a resolution of 1 cm^{-1} .

2.6. DPPH free radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging properties of the products were evaluated using the method of Wei *et al.* (2017). Samples at 125, 250, 500, and 1000 $\mu\text{g/ml}$ were combined with 2 mL of a methanolic DPPH solution (180 $\mu\text{mol/L}$) and incubated for 30 minutes at room temperature. The residual DPPH radical's absorbance was then measured at 517 nm in relation to a blank. Each sample was evaluated with three duplicates, and the antiradical effect was determined using the following equation:

$$\text{Percentage inhibition (\%)} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}} \times 100$$

2.7. Inhibition of protein denaturation assay

The inhibitory activity of bovine serum albumin denaturation was exploited in the study by Hu *et al.* (2020). 50 μL of coproduct oil (125, 250, 500, and 1000 $\mu\text{g/ml}$) and 950 μL of a bovine serum albumin solution (dissolved in 0.01 M PBS, pH 7.4, 1%, w/v) were used to create the reaction solution. In order to induce protein denaturation, all reaction solutions were stabilized at 37 °C for 15 minutes and then heated to 90 °C for 20 minutes. At room temperature (25 °C), 660 nm was used to measure turbidity. As a control, distilled water was used rather than the sample. The rate of denaturation inhibition was determined using the following formula:

$$\text{Percentage inhibition (\%)} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}} \times 100$$

2.8. Statistical analysis

Values were reported as mean $\pm$ SD (Standard Deviation) and each assay was performed in triplicate. Data were investigated with ANOVA analysis. The differences were recognized as significant at $P < 0.05$. The application of ANOVA was performed using the ANOVA function in R (R Core Team, 2024). The HSD.test function, under the Agricolae package was used to apply the Tukey test.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analysis of oil from coproducts

The extraction yield recorded a value of $26.06 \pm 0.13\%$ for *S. pilchardus* oil and $28.01 \pm 0.34\%$ for *T. thynnus* oil. Table 1 presents the results obtained from the physicochemical analysis of *S. pilchardus* and *T. thynnus* coproduct oil. The results showed an acid number value equal to 1.8 ± 0.15 and 2.46 ± 0.18 for the coproduct oil from *S. pilchardus* and *T. thynnus*, respectively. Both values appeared lower than those of Nascimento *et al.* (2015) (5.4 ± 0.1). Table 1 shows a value for the saponification index equal to 187.18 ± 1.59 and 182.87 ± 1.13 for the coproduct oil from *S. pilchardus* and *T. thynnus*, respectively. Purnamayati *et al.* (2019) recorded a value of 189.10 ± 1.61 for the saponification index of sardine oil. The ratio of long-chain fatty acids to short-chain fatty acids affected the level of saponification value. A quantity of long-chain fatty acids in the oil can decrease saponification.

TABLE 1. Physicochemical indexes of oil from coproducts

	Acid index (mg KOH/g)	Saponification index (mg KOH/g)	Peroxyde index (meq O ₂ /Kg)	Iodine index (g iodine/ 100 g of oil)	Relative density
Sardine	1.8 ± 0.15 ^a	187.18 ± 1.59 ^a	3.8 ± 0.25 ^a	121 ± 2 ^a	0.94 ± 0.008 ^a
Tuna	2.46 ± 0.18 ^b	182.87 ± 1.13 ^b	2.93 ± 0.08 ^b	119.83 ± 4.91 ^b	0.87 ± 0.011 ^b

Values are presented as mean ± SD of three measurements. Statistical significance was assessed by one-way analysis of variance ANOVA coupled with Tukey's post hoc test. According to Tukey's post hoc test, different letters (a, b, c, d, and e) in a column indicate a significant difference ($p < 0.05$) between groups.

Among the oil quality parameters is the peroxide index. This parameter makes it possible to evaluate the oil's rancidity by monitoring hydroperoxide formation. International fish oil standards require a peroxide value of 3.75 meq O₂/kg. The peroxide index obtained for *T. thynnus* oil was 2.93 ± 0.08 meq O₂/kg, which was comparable to the values obtained by Messina *et al.* (2022) in oil obtained from tuna coproducts. They showed a value of 2.96 ± 0.55 meq O₂/kg. Tuna oil may be suitable for human consumption if it has a peroxide value below 5 meq O₂/kg. A high peroxide value indicates that the oil has been oxidized by forming new peroxides through degradation to other compounds (Suseno *et al.*, 2015). Fish oil contains a large amount of unsaturated fatty acids. It, therefore, oxidizes quickly and becomes rancid. Prolonged rancidity forms peroxide and decreases fish quality by forming aldehydes or ketones as hydroperoxide coproducts (Pravinkumar *et al.*, 2015).

The iodine value expresses the degree of unsaturation in the oil associated with unsaturated fatty acids in oil or fat. The iodine value for fish oil was approximately 119–129 g I₂/100 g (Nascimento *et al.*, 2015). According to Table 1, the iodine value obtained was 121 ± 2 and 119.83 ± 4.91 g I₂/100 g for the coproduct oil from *S. pilchardus* and *T. thynnus*. Purnamayati *et al.* (2019) recorded a value of 123.52 ± 0.43 g I₂/100 g for the iodine value of sardine oil. For relative density, the coproduct oil from *S. pilchardus* and *T. thynnus* revealed a value of 0.94 ± 0.008 and 0.87 ± 0.011 , respectively. The value obtained was almost equal to that of Purnamayati *et al.* (2019), where they recorded a value of 0.93.

3.2. UV-Vis spectrophotometric analysis of oil

Spectrophotometric (UV-Vis) analysis of *S. pilchardus* and *T. thynnus* oil exhibited absorbances in wavelengths ranging from 200 to 800 nm (figure 1). The UV-visible absorption spectra presented a strong absorbance band at 310 nm. According to Oliveira *et al.* (2020), the spectra of omegas present characteristic absorption bands: $\lambda \approx 298$ nm for ω -3, $\lambda \approx 278$ nm for ω -6, λ equal to 227 and 291 nm for ω -9.

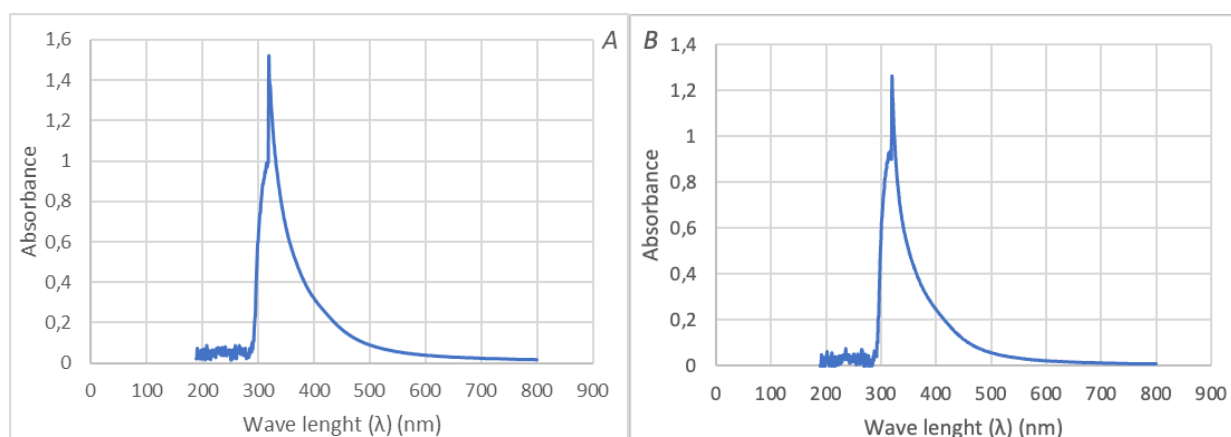


FIGURE 1. UV-Vis absorption spectrum of *S. pilchardus* (A) and *T. thynnus* (B) oil.

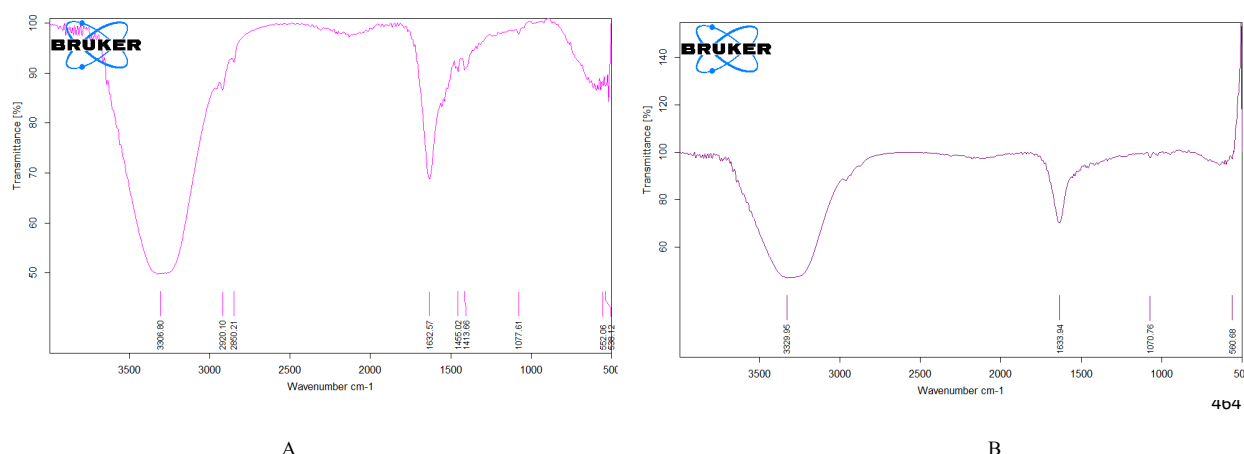


FIGURE 2. FTIR spectra of oil extracted from coproducts of *S. pilchardus* (A) and *T. thynnus* (B).

Therefore, the oil from *S. pilchardus* and *T. thynnus* may predominate ω -3 and 9 in its spectrum. The absorption band at $\lambda \approx 300$ nm can be attributed to tocopherols and unsaturated fatty acids (oleic, linoleic, linolenic). Additionally, Saito *et al.* (2023) found that fatty acids (oleic and linoleic acids) contributed significantly to UV absorption above 270 nm. Fully conjugated long-chain unsaturated fatty acids like linolenic acid (C18:3 Δ *cis*-9, *cis*-12, *cis*-15) have a strong and broad absorption band at $\lambda \approx 300$ nm (Lechuga and Michaelian, 2023). The primary dietary sources of EPA and DHA are fatty marine fish such as sardines. However, their high content of mono, di, and tri-unsaturated fatty acids was linked to the consumption of marine phytoplankton. Oleic acid (monounsaturated) was then exported to the endoplasmic reticulum and then desaturated by Δ 12 and Δ 15 desaturases, generating linoleic acid and α -linolenic acid, which served as precursors for ω -6 and ω -3 long chains (Jesionowska *et al.*, 2023).

3.3. Spectroscopic analysis (FTIR-ATR)

For the oil extracted from sardine and tuna coproducts, the FTIR-ATR spectrum presented a broadband at 3306 and 3329 cm⁻¹ which was linked to the vibration of C–H and the stretching of the *cis*-alkene -HC = CH - polyunsaturated fatty acids (Dórame-Miranda *et al.*, 2021). The high frequency of this band indicated the abundance of polyunsaturated acyl groups. A weak peak at 2920 cm⁻¹ for sardine represented the stretching vibration of -CH, -CH₂, and -CH₃ from an aliphatic alkane. An average peak appeared at 1632 and 1633 cm⁻¹, indicating elongation vibrations by the *cis* double bond (C=C). A weak peak appeared at 1077 and 1070 cm⁻¹, indicating the C=O of the COOH carboxylic group. A weak peak appeared at 552 and 560 cm⁻¹, representing the -HC=CH *cis* group.

However, no peaks representing secondary products of lipid oxidation (carbonyl group of aldehydes and ketones) were detected by FTIR spectra in the 3800–3400 cm⁻¹ range. There is evidence that during more prolonged extraction, the ester bond of triacylglycerols was cleaved by hot water (Pudtikajorn and Benjakul, 2020). In the study by Pudtikajorn and Benjakul (2020), the FTIR spectra of the tuna sample show that the main functional groups responsible for the IR absorption peaks were: C–H stretching vibration of the *cis* double bond at 3015 cm⁻¹; symmetric and asymmetric stretching vibration of CH₂ at 2920 and 2850 cm⁻¹, respectively. The results of the FTIR analysis carried out by Khalihena Groune *et al.* (2024) showed that sardine oil was very rich in polyunsaturated fatty acids (mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) and monounsaturated fatty acids (primarily oleic acid).

3.4. DPPH free radical scavenging assay

The antioxidant activity profile showed that the fractions tested have a dose-dependent anti-radical activity; that is to say, the increase in the concentration increased the percentage of inhibition (Table 2). The

anti-radical capacity was determined by comparison with the activity of a reference antioxidant compound (ascorbic acid). From the results obtained, it was noticed that for the concentration of 1000 µg/ml, the oil from *S. pilchardus* coproducts showed the highest percentage reduction ($89.96 \pm 0.29\%$) compared to *T. thynnus* ($83.72 \pm 2.08\%$). It should be mentioned that ascorbic acid remains more active than the fractions tested, with an inhibition percentage of $96.63 \pm 0.98\%$. The concentration necessary to inhibit 50% of the radical DPPH was calculated by linear regression of the inhibition percentages calculated as a function of different concentrations of the extracts. The IC_{50} values for the coproduct oil from *S. pilchardus* and *T. thynnus* are shown in Table 2.

TABLE 2. Anti-radical activity (%) of oil

	125	250	500	1000	IC_{50} (mg/ml)
Sardine	38.24 ± 0.22^a	58.85 ± 0.15^a	75.62 ± 0.35^a	89.96 ± 0.29^a	0.185
Tuna	27.19 ± 0.87^{ab}	52.62 ± 0.86^{ab}	72.03 ± 0.59^{ab}	83.72 ± 2.08^{ab}	0.2
Ascorbic acid	46.11 ± 0.39^d	69.90 ± 1.98^c	83.21 ± 2.35^b	96.63 ± 0.98^a	0.125

Values are presented as mean $\pm$ SD of three measurements. Statistical significance was assessed by one-way analysis of variance ANOVA coupled with Tukey's post hoc test. According to Tukey's post hoc test, different letters (a, b, c and d) in a column indicate a significant difference ($p < 0.05$) between groups.

The oil from *S. pilchardus* presented the highest antioxidant activity compared to *T. thynnus*, with an IC_{50} value equal to 0.185 mg/ml and 0.2 mg/ml, respectively. However, ascorbic acid showed the lowest IC_{50} value (0.125 mg/ml). Radical scavenging assays demonstrated that *S. pilchardus* lipids effectively neutralize peroxide, hydroxyl, and nitric oxide radicals. In a study by Borges *et al.* (2014), concentrations of 0.5 and 4 mg/ml were tested to evaluate the protective role of sardine oil in situations of induced oxidative stress. The results showed the increased protective effect of sardine oil rich in omega 3 against this oxidative stress.

Yates *et al.* (2014) showed that *Sardina pilchardus* oil reduced oxidative stress and neuroinflammation in several ways. Additionally, it was shown that this oil showed a dose-dependent inhibitory effect on DPPH radical scavenging activity. The maximum inhibition exhibited by the oil was $96.87 \pm 0.76\%$ at 4 mg/ml, and the IC_{50} was 0.148 mg/ml. Collective evidence suggested that the ratio of ω -3 PUFA is an essential dietary factor in reducing oxidative stress (Maffei *et al.*, 2014).

According to the results of Cardeira *et al.* (2022), the coproducts of *S. pilchardus* exhibited the highest ORAC value (1.94 ± 0.08 µmol TEAC/mg) in the radical absorption capacity test of oxygen. These results were consistent with a previous evaluation of antioxidants by an alternative method (2,2-diphenyl-1-picrylhydrazyl-DPPH), where the coproduct extracts presented the lowest IC_{50} values (Melgosa *et al.*, 2020). According to Trilaksani *et al.* (2023), the antioxidant activity of tuna oil produced IC_{50} values, namely F0 of 184.07 ppm, F1 of 119.63 ppm, and F2 of 130.45 ppm.

3.5. Inhibition of protein denaturation assay

According to the results (Table 3), there was a proportional relationship between the concentration of the sample and the percentage of inhibition of protein denaturation. In addition, it was shown that *S. pilchardus* oil presented a percentage of inhibition of BSA denaturation ranging from 33.23 ± 0.44 to $83.16 \pm 0.14\%$ for concentrations varying from 125 to 1000 µg/ml. It was followed by *T. thynnus* oil with a value of $81.09 \pm 0.4\%$ for the concentration 1000 µg/ml. The comparison with Ibuprofen showed that the latter presented a higher percentage of inhibition ($91.10 \pm 2.1\%$). The study by Yates *et al.* (2014) showed that the anti-inflammatory effect of *S. pilchardus* oil was more marked after 12 hours of incubation. The ability of *S. pilchardus* oil to help reduce inflammation was comparable to that of piroxicam (an anti-inflammatory drug), which was even more effective than the drug after 12 and 24 hours. Evidence suggested that DHA and EPA can be converted into anti-inflammatory eicosanoids and docosanoids called resolvins, protectins, and maresins. Fatty acids can also be directly converted to N-acyl ethanolamines (NAEs), which have anti-inflammatory properties (Balvers *et al.*, 2010).

TABLE 3. The inhibition percentage (%) of BSA by oil

	125	250	500	1000
Sardine	33.23 ± 0.44 ^d	61.71 ± 0.48 ^c	71.53 ± 0.53 ^b	83.16 ± 0.14 ^{ab}
Tuna	30.14 ± 0.21 ^d	59.28 ± 0.28 ^c	75.24 ± 0.63 ^b	81.09 ± 0.4 ^{ab}
Ibuprofen	36.78 ± 0.38 ^a	60.98 ± 0.3 ^a	78.02 ± 1.11 ^b	91.10 ± 2.1 ^c

Values are presented as mean ± SD of three measurements. Statistical significance was assessed by one-way analysis of variance ANOVA coupled with Tukey's post hoc test. According to Tukey's post hoc test, different letters (a, b, c and d) in a column indicate a significant difference ($p < 0.05$) between groups.

According to the study by Azeem *et al.* (2010), *Thunnus* coproduct extract showed significant anti-inflammatory activity, which may be due to the inhibition of inflammatory mediators by the action of steroids. Synergistic effects between hydrophobic and hydrophilic chemicals from different fish sources and anti-inflammatory qualities have been observed by other authors. Rudkowska *et al.* (2010) found that fish proteins and $\omega 3$ had a synergistic positive influence. According to Chevrier *et al.* (2015), the combination treatment resulted in a greater reduction of TNF- α expression compared to the treatment with $\omega 3$ alone.

4. CONCLUSIONS

All of these results showed that the oil from the coproducts of bluefin tuna (*Thunnus thynnus*) and sardine (*Sardina pilchardus*) have an interesting anti-radical and anti-inflammatory activity and, therefore, present considerable therapeutic interest as an alternative compound or therapeutic support for the prevention of inflammation, oxidative stress and cardiovascular pathologies. Future research should focus on optimizing the oil extraction processes, identifying specific bioactive lipid compounds, analyzing the fatty acid profiles, and evaluating their mechanisms of action in vitro and in vivo to better understand their therapeutic potential.

RESEARCH DATA POLICY

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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DECLARATION OF COMPETING INTEREST

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AUTHORSHIP CONTRIBUTION STATEMENT

H. Belkhodja, A. Remil, D. Bouhadi.: Conceptualization, Formal analysis, H. Belkhodja, A. Remil, F.Z. Labbaci, F. Belkhadem: Investigation, Methodology, H. Belkhodja, A. Remil, Z. Benattouche: Project administration, Writing – original draft, Writing – review & editing.

Artificial Intelligence Usage

The authors of this article declare that there is no artificial intelligence usage.

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