

Fatty acids profile and anticholinesterase activity of fish lipids from Brazilian Northeast

Perfil de ácidos graxos e atividade anticolinesterásica de lipídios de peixes do Nordeste do Brasil

Perfil de ácidos grasos y actividad anticolinesterasa de los lípidos de pescado del Noreste de Brasil

Received: 08/02/2021 | Reviewed: 08/08/2021 | Accept: 08/12/2021 | Published: 08/15/2021

Séfura Maria Assis Moura

ORCID: <https://orcid.org/0000-0002-7968-7874>

Federal Institute of Education, Science and Technology of Ceará, Brazil

E-mail: sefura@ifce.edu.br

Selene Maia de Moraes

ORCID: <https://orcid.org/0000-0002-4000-7616>

State University of Ceará, Brazil

E-mail: selenemaiademoraes@gmail.com

José Osvaldo Beserra Carioca

ORCID: <https://orcid.org/0000-0002-8086-011X>

Federal University of Ceará, Brazil

E-mail: jkriok@gmail.com

Ana Livya Moreira Rodrigues

ORCID: <https://orcid.org/0000-0003-1177-1233>

State University of Ceará, Brazil

E-mail: livya_rodrigues@hotmail.com

Daniela Ribeiro Alves

ORCID: <https://orcid.org/0000-0002-0746-2211>

State University of Ceará, Brazil

E-mail: alves.danielaribeiro@gmail.com

Francisco Felipe Maia da Silva

ORCID: <https://orcid.org/0000-0001-6794-109X>

Federal Institute of Education, Science and Technology of Rio Grande do Norte, Brazil

E-mail: felipe.maia@ifrn.edu.br

Ana Carolina Silva e Silva

ORCID: <https://orcid.org/0000-0002-1808-7437>

State University of Ceará, Brazil

E-mail: carolinaana1310@gmail.com

Sheyla Maria Barreto Amaral

ORCID: <https://orcid.org/0000-0003-0041-5487>

Federal Institute of Education, Science and Technology of Ceará, Brazil

E-mail: sheylaamaral82@gmail.com

Ysabele Yngrydh Valente Silva

ORCID: <https://orcid.org/0000-0002-8500-1525>

Federal Institute of Education, Science and Technology of Ceará, Brazil

E-mail: ysabelevalentin@gmail.com

Abstract

Acetylcholine deficiency is a neurochemical characteristic of patients with clinical diagnosis of Alzheimer's disease. Substances that inhibit the enzyme acetylcholinesterase, increasing levels of acetylcholine in the brain, are a promising form of treatment. Studies relate the use of omega-3 fatty acids in the treatment and prevention of Alzheimer's disease. The Northeast Region of Brazil has an enormous biological diversity and a wide variety of fish species. In this work, the oils of eleven species of marine fish found on the coast of Ceará, Brazil, were analyzed in relation to the fatty acid profile and the inhibitory activity of the enzyme acetylcholinesterase. Total lipids were extracted from fish samples by Folch methodology. The lipid extracts of the fish and industrialized fish oil, used for comparison, were esterified and fatty acid profiles were analyzed. The acetylcholinesterase inhibitory activity was measured quantitatively. The oils presented a high percentage of saturated fatty acids, which is a general characteristic of tropical fish. Oleic acid was the highest monounsaturated fatty acid. Oils of *Scomberomorus cavalla*, *Lutjanus synagris* and *Haemulon plumieri* presented expressive percentages of polyunsaturated fatty acids and the most potent anticholinesterase activities. This research showed the oils of *S. cavalla*, *L. synagris* and *H. plumieri* may be promising functional food products of active fatty acids as new therapies to treatment or prevention of Alzheimer's disease. The expressive concentration of unsaturated and polyunsaturated fatty acids together with their relevant anticholinesterase activity are characteristics of the importance of these fish oils.

Keywords: Marine fish; Fatty acids composition; Polyunsaturated fatty acids; Acetylcholinesterase; Alzheimer's disease.

Resumo

A deficiência da acetilcolina é uma característica neuroquímica de pacientes com diagnóstico clínico da doença de Alzheimer. As substâncias que inibem a enzima acetilcolinesterase, aumentando os níveis de acetilcolina no cérebro, são uma forma promissora de tratamento. Estudos relacionam o uso de ácidos graxos ômega-3 no tratamento e prevenção da doença. Neste trabalho, os óleos de onze espécies de peixes marinhos encontrados no litoral do Ceará, Brasil, foram analisados em relação ao perfil de ácidos graxos e à atividade inibitória da enzima acetilcolinesterase. Os lipídios totais foram extraídos das amostras de peixes pela metodologia de Folch. Os extratos lipídicos dos peixes e o óleo de peixe industrializado, usado para comparação, foram esterificados e os perfis de ácidos graxos analisados. A atividade inibitória da acetilcolinesterase foi medida quantitativamente. Os óleos apresentaram elevados percentuais de ácidos graxos saturados, característica dos peixes de águas tropicais. O ácido oléico representou o ácido graxo monoinsaturado em maior proporção. Os óleos de *Scomberomorus cavalla*, *Lutjanus synagris* e *Haemulon plumieri* apresentaram percentuais expressivos de ácidos graxos poli-insaturados, assim como as mais potentes atividades anticolinesterásicas. Dessa forma, a pesquisa demonstrou a importância dos óleos de *S. cavalla*, *L. synagris* e *H. plumieri*, como alimentos funcionais ricos em ácidos graxos ativos, e que podem ser utilizados em novas terapias para o tratamento ou prevenção da doença de Alzheimer.

Palavras-chave: Peixes marinhos; Composição de ácidos graxos; Ácidos graxos poli-insaturados; Acetylcolinesterase; Doença de Alzheimer.

Resumen

La deficiencia de acetilcolina es una característica neuroquímica de los pacientes con diagnóstico clínico de enfermedad de Alzheimer. Las sustancias que inhiben la enzima acetilcolinesterasa, aumentando los niveles de acetilcolina en el cerebro, son una forma prometedora de tratamiento. Los estudios relacionan el uso de ácidos grasos omega-3 al tratamiento y prevención de la enfermedad. En este trabajo, se analizaron los aceites de once especies de peces marinos que se encuentran en el litoral del Ceará, Brasil, en relación con el perfil de ácidos grasos y la actividad inhibidora de la enzima acetilcolinesterasa. Los lípidos totales se trajeron de muestras de pescado utilizando la metodología de Folch. Se esterificaron extractos de lípidos de pescado y aceite de pescado industrializado, utilizados como comparación, y se analizaron los perfiles de ácidos grasos. La actividad inhibidora de la acetilcolinesterasa se midió cuantitativamente. Los aceites presentaron altos porcentajes de ácidos grasos saturados, característicos de los pescados de aguas tropicales. El ácido oleico representó al ácido graso monoinsaturado en mayor proporción. Los aceites de *Scomberomorus cavalla*, *Lutjanus synagris* y *Haemulon plumieri* presentaron expresivos porcentajes de ácidos grasos poliinsaturados, así como las actividades anticolinesteras más potentes. Así, la investigación demostró la importancia de los aceites de *S. cavalla*, *L. synagris* y *H. plumieri*, alimentos funcionales ricos en ácidos grasos activos y que pueden utilizarse en nuevas terapias para el tratamiento o prevención de la enfermedad de Alzheimer.

Palabras clave: Pescado marino; Composición de ácidos grasos; Ácidos grasos poliinsaturados; Acetylcolinesterasa; Enfermedad de Alzheimer.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease with a slow and continuous progression affecting mainly the elderly population of the world (over 65 years of age). It has become increasingly worrying in view of the increase in life expectancy. It is the most common cause of dementia. Its characteristics are difficulty in memory, language, problem solving and other cognitive abilities that affect one's ability to perform everyday activities (Alzheimer's Association, 2018).

Several observational epidemiological studies have pointed to the benefits of omega 3, especially at the early stages of AD (Barberger-Gateau et al., 2007; Huang et al., 2005; Wu et al., 2015).

In addition to a regular consumption of fish, the supplementation with omega 3 fatty acids as a possibility of prevention or deceleration of the disease has been investigated in moderate clinical settings. Such studies relate oils mainly to persistent neuroinflammatory processes in the patients' brains (Fraga, Carvalho, Caramelli, Sousa & Gomes, 2017; Hooijmans, Jong, Vries & Ritskes-Hoitinga, 2012; Kerdiles, Lay & Calon, 2017; Song, Shieh, Wu, Kalueff, Gaikwad & Su, 2016).

For the brain, the beneficial effects of omega 3 fatty acids are numerous, especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. The most cited benefits are participation in the structure and functions of the cell membrane,

modulation of inflammation with production of eicosanoids, regulation of immune function, and action on oxidative stress and gene expression (Calder & Grimble, 2002; Calon et al., 2004; Vedin et al., 2012; James, Gibson & Cleland, 2000).

According to the Alzheimer's Association (2018), several changes occur to the brain during the development of the disease. Such changes may result from different factors.

Among the changes observed, decreased quantities of DHA, one of the most abundant fatty acids in a healthy brain, are found in AD patients, especially in the most affected regions (Belkouch et al., 2016; Calviello, Serini & Piccioni, 2008).

Acetylcholine deficiency is also a neurochemical characteristic of clinically diagnosed patients. Acetylcholine is one of the major neurotransmitters by which electrical impulses are driven through nerve cells and transmitted to other nerve cells or to voluntary and involuntary muscles (Elufioye, Obuotor, Sennuga, Agbedahunsi & Adesanwa, 2010).

Substances that inhibit the enzyme acetylcholinesterase by increasing the levels of acetylcholine in the brain are a promising form of AD treatment (Huang, Su & Li, 2013).

Some drugs have been used for inhibiting this enzyme. On the other hand, natural products such as extracts and substances isolated from plants, algae and other marine organisms have been widely investigated (Bianco et al., 2015; Morais et al., 2017; Penido et al., 2017).

Substances or materials that have a positive effect on cognitive disorders, whether by anti-inflammatory, antioxidant or acetylcholinesterase inhibitory action or by two or more mechanisms, are of great interest for the treatment of AD (Belkouch et al., 2016; Penido et al., 2017).

The Brazilian Northeastern Region has an enormous biological diversity and a great variety of fish species. Fish oils can be investigated as for its pharmacological and biotechnological potential. Thus, the oils of eleven fish species commonly found in the state of Ceará, Northeastern Region of Brazil, were analyzed and an industrialized fish oil commercialized in pharmacies rich in omega 3 fatty acids, was used for comparison. The objective of this work is to evaluate which types of fish oils have the greatest inhibiting action of the enzyme acetylcholinesterase to value local fishes.

2. Methodology

The present study refers to an experimental qualitative research, with numerical data obtained in the laboratory (Pereira et al., 2018)

2.1 Collection and preparation of samples

Fish samples were obtained near the fish market in Fortaleza, Ceará, Northeast of Brazil, from local anglers. Three fresh specimens of each species of sea fishes were purchased. The samples were transported to the laboratory in iceboxes. The heads were removed and the fillets were separated and milled in a processor until a homogeneous mass was formed. This mass was used for analysis the species analyzed were *Balistes capiscus* (grey triggerfish), *Cephalopholis fulva* (coney), *Ginglymostoma cirratum* (nurse shark), *Haemulon aurolineatum* (tomtate grunt), *Haemulon plumieri* (white grunt), *Lutjanus synagris* (lane snapper), *Malacanthus plumieri* (sand tilefish), *Myripristes jacobus* (blackbar soldierfish), *Scomberomorus brasiliensis* (serra Spanish mackerel), *Scomberomorus cavalla* (king mackerel) and *Spalisoma chrysopterum* (redtail parrotfish).

Industrialized fish oil was purchased from local stores, in a bottle containing 60 capsules, for comparison.

2.2 Materials

All reagents and solvents were of analytical grade.

2.3 Lipid extraction

Total lipids were extracted from fish samples using the method of Folch (Folch, Lees & Stanley, 1957) in 100 g aliquots of samples and a mixture of chloroform/methanol (3:1).

2.4 Transmethylation of fish lipids

The lipid extracts were esterified according to the methodology of the International Union of Pure and Applied Chemistry (International Union of Pure and Applied Chemistry [IUPAC], 1987). The method comprises mixing 500 mg of lipids with hexane (5 mL) and KOH at 0.1M into methanol (5 mL) in a capped test tube heated in 50°C water bath for one hour with a subsequent separation of methyl esters.

2.5 Determination of fatty acid profiles GC/MS

Fatty acid profiles were analyzed by gas chromatography coupled to a mass spectrometer - Shimadzu Q P-2010 instrument with a DB-5ms fused silica capillary column containing dimethylpolysiloxane (30 m x 0.25 mm x 0.25 µm), drag gas: He (1 mL/min) in constant linear velocity mode, injection temperature: 250°C, and detector temperature: 200°C. The temperature of the column was set from 35 to 180°C at 4°C/min, from 180 to 280°C at 17°C/min and at 280°C for 10 min. The mass spectrum was obtained by electron impact at 70 eV. The compounds were identified by retention times, comparison of the mass spectra obtained with spectra recorded in a database (National Institute of Standards and Technology database NIST: 147,198 compounds, USA), and by visual comparison with spectra published in the catalog of mass spectra (Adams, 2001).

2.6 Determination of acetylcholinesterase inhibitory activity,

The inhibitory activity of acetylcholinesterase was quantitatively measured according to the method described by Ellman, Courtney, Valentino and Featherstone (1961) modified by Trevisan, Macedo, Van Den Meent, Rhee and Verpoorte (2003) using an Elisa BIOTEK microplate reader (ELX 800 model with Gen5 software V2.04.11). In 96-well plates, the following solutions were used per well: 25 µL of acetylthiocholine iodide (15 mM), 125 µL of 5,5'-dithiobis-[2-nitrobenzoic] in Tris/HCl solution (50 mM, pH = 8), 0.1 M NaCl and 0.02 M MgCl₂.6H₂O (3 mM, DTNB or Ellman's reagent), 50 µL of Tris/HCl solution (50 mM, pH = 8), 0.1% of bovine serum albumin (BSA), 25 µL of the sample dissolved in ethyl acetate and diluted ten times in Tris/HCl solution (50 mM, pH = 8) to obtain a final concentration of 0.2 mg.mL⁻¹ (Rhee, Meent, Ingkaninan & Verpoorte, 2001).

Absorbance was measured at 405 nm for 30 seconds. Then, 25 µL of acetylcholinesterase enzyme was added and absorbance was measured per minute up to 25 minutes of enzyme incubation. As a negative standard, all solutions were used, except for the sample. The dilutions of the samples and the positive standards used in the quantitative microplate assays, starting from the 20 mg/mL concentration stock solution, were 200µg.mL⁻¹, 100 µg.mL⁻¹, 50 µg.mL⁻¹, 25 µg.mL⁻¹, 12.5 µg.mL⁻¹, 6.25 µg.mL⁻¹, 3.12 µg.mL⁻¹, 1.56 µg.mL⁻¹, and 0.78 µg.mL⁻¹.

The standard used as a positive control was Physostigmine.

All samples were analyzed in triplicate. After data normalization, a non-linear regression curve test was performed using the statistical software GraphPad Prism version 5.01.

2.7 Statistical Analysis

Data were expressed as mean ± standard deviation. Differences between values were examined using analysis of variance (ANOVA). The results were compared by Tukey test at a 5% significance level.

3. Results and Discussion

3.1 Fatty acid profile

The percentage of lipids (g/100 g) of the meat of different fish species varied between 0.10 and 4.80%, according to Table 1.

Table 1: Lipid content of marine eleven fish species from the state of Ceará/Brazil.

Species	% Lipids	Species	% Lipids
<i>Balistes capiscus</i>	0.10 ± 0.03 ^e	<i>Malacanthus plumieri</i>	1.54 ± 0.46 ^{cd}
<i>Cephalopholis fulva</i>	0.52 ± 0.21 ^e	<i>Myripristis jacobus</i>	0.73 ± 0.57 ^{de}
<i>Ginglymostoma cirratum</i>	4.80 ± 0.52 ^a	<i>Scomberomorus brasiliensis</i>	1.95 ± 0.00 ^{bc}
<i>Haemulon aurolineatum</i>	1.61 ± 0.28 ^{bc}	<i>Scomberomorus cavala</i>	2.42 ± 0.00 ^b
<i>Haemulon plumieri</i>	1.54 ± 0.46 ^{cd}	<i>Sparisoma chrysopterum</i>	0.08 ± 0.01 ^e
<i>Lutjanus synagris</i>	0.43 ± 0.15 ^e		

Data is presented as mean ± standard deviation and analyzed by ANOVA followed by Tukey test. Values with different superscript letters are significantly different ($p < 0,05$). Source: Authors.

All species could be classified as lean fishes because they had a fat content below 5% (Penfield & Campbell, 1990).

Oils of fish captured on the coast of Ceará showed a high percentage of saturated fatty acids (SFA) (Table 2). This is a characteristic of fish from warm waters of tropical zones like in the Brazilian Northeast seas (Dey, Buda, Wiik, Halver & Farkas, 1993).

Table 2: Fatty acids % composition of fillet oils of marine fish from Ceará/Brazil by gas chromatography coupled to mass spectrometry.

Fatty acids	Species										
	Bc	Cf	Gc	Ha	Hp	Ls	Mp	Mj	Sb	Sc	Sch
C14:0	2.84	5.31	5.05	4.91	4.71	5.28	5.90	4.23	7.34	2.60	4.34
C15:0	0.81	ND	1.08	1.47	0.90	0.95	1.77	1.39	1.01	ND	1.42
C16:0	45.30	40.56	31.04	43.69	26.66	33.30	42.98	31.61	34.60	26.92	37.03
C17:0	1.37	2.13	1.63	2.63	2.37	2.87	2.66	1.61	1.36	ND	7.32
C18:0	22.68	21.35	17.58	16.35	9.55	12.50	18.48	18.01	16.10	14.43	22.03
C19:0	0.40	ND	0.48	ND	1.31						
C20:0	0.73	ND	0.89	0.83	ND						
C22:0	0.33	3.09	0.71	0.43	ND	ND	1.20	7.28	ND	ND	ND
C24:0	ND	3.85	0.78	ND	ND	ND	0.75	3.90	0.67	ND	ND
C16:1n-7	2.46	3.58	7.43	4.68	12.21	8.86	3.38	3.22	8.59	6.11	6.62
C17:1n-7	0.23	ND	ND	ND	0.59	0.78	ND	ND	ND	ND	ND
C18:1n-9c	21.68	14.79	28.47	21.64	34.95	25.66	15.03	15.08	27.26	32.17	21.59
C18:1n-9t	ND	ND	ND	ND	ND	ND	2.47	ND	ND	ND	ND
C20:1n-9	ND	1.38	1.99	2.78	ND	ND	2.01	2.71	ND	1.87	0.75
C20:1n-7	0.85	ND	0.55	0.28	0.57	1.12	ND	ND	1.28	ND	ND
C22:1n-9	ND	ND	ND	ND	ND	ND	0.60	5.35	ND	ND	ND
C24:1n-9	0.32	2.30	0.87	ND	ND	ND	0.81	4.16	0.91	ND	ND
C18:2n-6	ND	ND	0.91	ND							
C20:2n-6	ND	1.66	ND	0.31	ND	ND	ND	1.45	ND	ND	ND
C20:4n-6	ND	ND	0.54	ND	2.66	2.56	ND	ND	ND	ND	ND
C20:5n-3	ND	ND	ND	ND	2.10	1.86	ND	ND	ND	5.66	ND
C22:4n-6	ND	ND	ND	ND	0.49	0.66	ND	ND	ND	ND	ND
C22:5n-3	ND	ND	ND	ND	0.28	ND	ND	ND	ND	ND	ND
C22:6n-3	ND	ND	ND	ND	1.96	3.60	ND	ND	ND	10.24	ND
Total SFA	74.46	76.29	59.24	70.31	44.19	54.90	75.19	68.03	61.96	43.95	71.04
Total MUFA	25.54	22.05	39.31	29.39	48.32	36.42	24.30	30.52	38.04	40.15	28.96
Total PUFA	ND	1.66	1.45	0.30	7.49	8.68	ND	1.45	ND	15.90	ND

c = cis and t = trans; Bc=*Balistes capiscus*; Cf=*Cephalopholis fulva*; Gc=*Ginglymostoma cirratum*; Ha=*Haemulon aurolineatum*; Hp=*Haemulon plumieri*; Ls=*Lutjanus synagris*; Mj=*Myripristis jacobus*; Mp=*Malacanthus plumieri*; Sb=*Scomberomorus brasiliensis*; Sc=*Scomberomorus cavalla*; Sch=*Sparisoma chrysopterum*; SFA=Saturated fatty acids; MUFA=Monounsaturated fatty acids; PUFA=Polyunsaturated fatty acids; ND=Not Determined. Source: Authors.

Palmitic acid (16:0) was the most concentrated saturated fatty acid (SFA) in most oils, ranging from 26.66% to 45.30%. In the analysis of lipids of sea fish, other studies also mention palmitic acid as the main constituent among SFA (Andrade, Bispo & Druzian, 2009; Ozogul & Ozogul, 2007; Ozogul, Ozogul & Alagoz, 2007; Prato & Biantolino, 2012).

Among the eleven oils analyzed, the ones with the highest concentrations of polyunsaturated fatty acids (PUFA) were the oils of *S. cavalla*, *L. synagris* and *H. plumieri*. Other studies reported high percentages of PUFA in fish caught in Brazilian Northeastern waters. However, the results for these three species show that they are good sources of PUFA. Fernandes et al. (2014) analyzing ballyhoo halfbeak (*Hemiramphus brasiliensis*), common halfbeak (*Hyporhamphus unifasciatus*), Atlantic thread herring (*Opisthonema oglinum*) and king mackerel (*Scomberomorus cavalla*), found percentages of PUFA ranging from 20.26% to 48.81%. By analyzing striped mojarra (*Eugerres plumieri*) and king mackerel (*Scomberomorus cavalla*), Menezes et al. (Menezes, Lira, Mena & Sant'ana, 2009) obtained PUFA percentages of 24.71% and 40.89%, respectively.

The oleic acid (18:1) was the monounsaturated fatty acid (MUFA) found at the highest proportion in all oils, ranging from 14.79% to 34.95%. These results are higher than the results reported by Prato and Biantolino (2012), who analyzed eleven commercially important fish species from the Mediterranean Sea, south of Italy. The values ranged from 5.52% to 13.87%. De Almeida and Silva (Almeida & Silva, 2016) found percentages of oleic acid ranging from 8.97% to 17.82% for three species of the Lutjanidae family in the Atlantic Coast of the Amazon.

The fatty acid profile of commercially available industrialized fish oil is shown in Table 3.

Table 3: Percentage composition of fatty acids of commercial fish oil.

Fatty acids	%	Fatty acids	%
C14:0	6.92	C18:2	1.53
C16:0	17.48	C18:2	0.37
C17:0	0.49	C18:3 (ω-3)	1.40
C18:0	3.89	C16:4 (ω-3)	1.85
C19:0	1.63	C20:4 (ω-3)	2.05
C20:0	0.40	C20:4	3.11
C16:1	10.23	C20:5 (ω-3) EPA	19.80
C18:1	15.22	C22:5 (ω-3)	0.41
C20:1	1.35	C22:6 (ω-3) DHA	11.85

Total Saturated Fatty Acids: 30.81 %
Total Monounsaturated Fatty Acids: 26.80 %
Total Polyunsaturated Fatty Acids: 42.37 %

Source: Authors.

In relation to commercially available fish oil, palmitic acid and oleic acid are also presented as the main constituents of SFA and MUFA, but at lower concentrations than most other fish oils, especially in relation to saturated acids. With regard to PUFA, the content was expected since it is a fish oil with a high content of omega 3 fatty acids.

3.2 Inhibitory activity of the acetylcholinesterase enzyme

The results obtained in the inhibition of AChE activity were compared to that of the alkaloid physostigmine, which was the first discovered natural inhibitor. Santos et al. (2018), determined the anticholinesterase activity of extracts and fractions from 54 plants and classified the action according to the IC₅₀ values as: high potency when IC₅₀ < 20 µg. mL⁻¹. Evaluating the results obtained in the AChE inhibition test, it is observed that all fish oils presented results below 20 µg. mL⁻¹, therefore with a high inhibition power. Then, all oils extracted from regional fish showed good results for acetylcholinesterase

inhibitory activity (AChEI) (Table 4), with emphasis on the oils of the species *S. cavalla*, *L. synagris* and *H. plumieri*, whose values of IC₅₀ (the concentration that inhibited 50% of the activity of the enzyme) were the lowest, corresponding to a greater bioactivity.

Table 4: Acetylcholinesterase inhibitory activity of fish fillet oils in state of Ceará/Brazil and commercial fish oil.

Samples	(IC ₅₀ µg/ mL)
<i>Balistes capiscus</i>	8.73 ± 0.075 ^c
<i>Cephalopholis fulva</i>	7.09 ± 0.040 ^d
<i>Ginglymostoma cirratum</i>	11.32 ± 0.011 ^a
<i>Haemulon aurolineatum</i>	7.74 ± 0.065 ^d
<i>Haemulon plumieri</i>	4.81 ± 0.029 ^e
<i>Lutjanus synagris</i>	2.84 ± 0.044 ^f
<i>Malacanthus plumieri</i>	7.97 ± 0.037 ^d
<i>Myripristis jacobus</i>	11.69 ± 0.003 ^a
<i>Scomberomorus brasiliensis</i>	11.10 ± 0.029 ^a
<i>Scomberomorus cavala</i>	2.60 ± 0.150 ^f
<i>Sparisoma chrysopterum</i>	8.81 ± 0.046 ^b
Comercial fish oil	4.37 ± 0.800 ^e
Physostigmine (Control)	1.15 ± 0.047 ^g

Date are presented as mean ± standard deviation and analyzed by ANOVA followed by Tukey test. Values with different superscript letters are significantly different (P < 0.05). Medium acetylcholinesterase Inhibition (AChEI): IC₅₀ (µg/mL). Source: Authors.

Oils with high concentrations of PUFA had the best anticholinesterase activity, including the oil used therapeutically.

The oils of *L. synagris* and *S. cavalla*, which had the best AChE inhibitory – IC₅₀ values, contain the highest concentrations of DHA among the oils with the best activity.

Some studies on plant oils and plant pulps with high percentages of unsaturated fatty acids (UFA), with emphasis on oleic and linoleic acids, also show results, ranging from moderate to potent in relation to percent inhibition of the AChE enzyme (Fernández et al., 2016; Itriago et al., 2017; Santos et al., 2015; Vinutha et al., 2007).

Polyunsaturated fatty acids, especially EPA and DHA, are among the most studied nutrients with regard to neuroprotective effects. They promote improvement of cognitive abilities and acts on brain neurodegenerative diseases. However, there is still no consensus on their effectiveness (Kerdiles, Lay & Calon, 2017; Wu, Ding, Wu, Hou & Mao, 2015;).

The efficient inhibitory activity of the acetylcholinesterase enzyme corroborates the potential of omega 3 fatty acids as they may increase acetylcholine concentration in the brain.

Moreover, a synergistic action between the PUFA anti-inflammatory process, cited by several authors, and inhibition of the enzyme should be evaluated in relation to the importance of PUFA and UFA for the treatment of AD-induced dysfunctions (Calder & Grimble, 2002; Mesquita et al., 2011).

4. Conclusion

In conclusion, this research indicated that the oils of *S. cavalla*, *L. synagris* and *H. plumieri* as functional food as new therapies to treat or prevent AD. They are promising due to an expressive concentration of UFA and PUFA together with a relevant anticholinesterase activity.

For future works, it is suggested to select fish samples with high levels of fatty acids for further analyses, such as carrying out tests with zebrafish and other models. It is also suggested to carry out new surveys with other fish species from the

Northeast Coast to find new study targets. Besides, it is important to encourage the population to consume the fish species that had the best results.

Acknowledgments

The authors acknowledge the financial support and fellowships granted from the Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological Development (CNPq), the Cearence Foundation to Support Scientific and Technological Development (FUNCAP) and Federal Institute of Education, Science and Technology of Ceara.

References

- Adams, R. P. (2001). *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. Carol Stream, IL: Allured Publishing Corporation.
- Almeida, E. S. & Silva, E. M. (2016). Chemometric identification and nutritional evaluation of three species of Lutjanidae (Perciformes) from the Amazonian Atlantic Coast based on fatty acid profiles. *Acta Amazonica*, 46 (4), 401–410. <https://dx.doi.org/10.1590/1809-4392201505254>
- Alzheimer's Association. (2018). *Alzheimer's Disease Facts and Figures*. *Alzheimers Dement*, 14(3), 367-429. <https://www.alz.org/media/homeofice/facts%20and%20figures/facts-and-figures.pdf>
- Andrade, G. Q., Bispo, E. S. & Druzian, J. I. (2009). Evaluation of nutritional quality in fish species most produced in the State of Bahia. *Ciência e Tecnologia de Alimentos*, 29(4), 721-26. <https://doi: 10.1590/S0101-20612009000400004>
- Barberger-Gateau, P., Raffaitin, C., Letenneur, L., Berr, C., Tzourio, C., Dartigues, J. F. & Alperovitch, A. (2007). Dietary patterns and risk of dementia: The Three-Citycohort study. *Neurology*, 69, 1921–30. <https://doi: 10.1212/01.wnl.0000278116.37320.52>
- Belkouch, M., Hachem, M., Elgot, A., Van, A. L., Picq, M., Guichardant, M., Lagarde, M., & Bernoud-Hubac, N. (2016). The pleiotropic effects of omega-3 docosahexaenoic acid on the hallmarks of Alzheimer's disease. *The Journal of Nutritional Biochemistry*, 38, 1–11. <https://doi: 10.1016/j.jnutbio.2016.03.002>
- Bianco, E. M., Krug, J. L., Zimath, P. L., Kroger, A., Paganelli, C. J., Boeder, A. M., Santos, L., Tenfena, A., Ribeiro, S. M., Kuroshima, K. N., Alberton, M. M. D., Cordova, C. M. M. & Rebelo, R.A. (2015). Antimicrobial (including antimollicutes), antioxidant and anticholinesterase activities of Brazilian and Spanish marine organisms – evaluation of extracts and pure compounds. *Revista Brasileira de Farmacognosia*, 25, 668–76. <https://doi.org/10.1016/j.bjfp.2015.07.018>
- Calder, P. C. & Grimble, R. F. (2002). Polyunsaturated fatty acids, inflammation and immunity. *European Journal Clinical Nutrition*, 56, 3(Suppl), S14-S19. <https://doi.org/10.1038/sj.ejcn.1601478>
- Calon, F., Lim, G. P., Yang, F., Moribara, T., Teter, B., Ubeda, O., Rostaing, P., Triller, A., Salem, N. Jr., Ashe, K. H., Frautschy, S. A. & Cole, G. M. (2004). Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron*, 43(5), 633-45. <https://doi.org/10.1016/j.neuron.2004.08.013>
- Calviello, G., Serini, S. & Piccioni, E. (2008). Alzheimer's Disease and n-3 Polyunsaturated Fatty Acids: Beneficial Effects and Possible Molecular Pathways Involved. *Current Signal Transduction Therapy*, 3(3), 152-57. <https://doi:10.2174/157436208785699659>
- Dey, I., Buda, C., Wiik, T., Halver, J. E. & Farkas, T. (1993). Molecular and structural composition of phospholipid membranes in livers of marine and freshwater fish in relation to temperature. *Proceedings of the National Academy Sciences of the United States of America*, 90 (16), 7498-7502. <https://doi: 10.1073/pnas.90.16.7498>
- Ellman, G. L. K., Courtney, D., Valentino, A. J. & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7 (2), 88-95. [http://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi:10.1016/0006-2952(61)90145-9)
- Elufioye, T. O., Obuotor, E. M., Sennuga, A. T., Agbedahunsi, J. M. & Adesanya, S.A. (2010). Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some selected Nigerian medicinal plants. *Revista Brasileira de Farmacognosia*, 20 (4),472–77. <https://doi.org/10.1590/S0102-695X2010000400002>
- Fernandes, C. A., Vasconcelos, M. A. S., Ribeiro, M. A., Sarubbo, L. A., Andrade, S. A.C. & Melo Filho, A. B. (2014). Nutritional and lipid profiles in marine fish species from Brazil. *Food Chemistry*, 160, 67–71. [http://doi:10.1016/j.foodchem.2014.03.055](https://doi:10.1016/j.foodchem.2014.03.055)
- Fernández, I. M., Mozombite, D. M. S., Santos, R. C., Melo Filho, A. A., Ribeiro, P. R. E., Chagas, E. A., Takahashi, J. A., Ferraz, V. P., Melo, A. C. G. R. & Maldonado, S. A. S. (2016). Oil in Inajá Pulp (*Maximiliana maripa*): Fatty Acid Profile and Anti-acetylcholinesterase Activity. *Orbital: The Electronic Journal of Chemistry*, 8 (2). <https://dx.doi.org/10.17807/orbital.v7i4.769>
- Fraga, V. G., Carvalho, M. G., Caramelli, P., Sousa, L. P. & Gomes, K. B. (2017) Resolution of inflammation, n-3 fatty acid supplementation and Alzheimer disease: A narrative review. *Journal Neuroimmunology*. 15(310), 111–19. [http://doi:10.1016/j.jneuroim.2017.07.005](https://doi:10.1016/j.jneuroim.2017.07.005)
- Folch, J., Lees, M. & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, 226 (1), 497-509. [https://doi.org/10.1016/s0021-9258\(18\)64849-5](https://doi.org/10.1016/s0021-9258(18)64849-5)

- Hooijmans, C. R., Jong, P. C. M. P., Vries, R. B. M. & Ritskes-Hoitinga, M. (2012) The Effects of Long-Term Omega-3 Fatty Acid Supplementation on Cognition and Alzheimer's Pathology in Animal Models of Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Journal of Alzheimer's Disease*, 28, 191–209. <http://doi: 10.3233/JAD-2011-111217>
- Huang, L., Su, T. & Li, X. (2013). Natural products as sources of new lead compounds for the treatment of Alzheimer's disease. *Current Topics in Medicinal Chemistry*, 13(15), 1864–1878. <https://doi: 10.2174/15680266113139990142>
- Huang, T. L., Zandi, P. P., Tucker, K. L., Fitzpatrick, A. L., Kuller, L., Fried, L. P., Burke, G. L. & Carlson, M. C. (2005). Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. *Neurology*, 65, 1409–14. <https://doi: 10.1212/01.wnl.0000183148.34197.2e>
- International Union of Pure and Applied Chemistry (1987). *Standard methods for the analysis of oils, fats and derivatives* (7th ed). Blackwell Scientific Publications.
- Itriago, C. V., Melo Filho, A. A., Ribeiro, P. R. E., Melo, A. C. G. R., Takahashi, J. A., Ferraz, V. P., Mozombite, D. M. S. & Santos, R. C. (2017). Inhibition of acetylcholinesterase and Fatty Acid Composition in Theobroma grandiflorum Seeds. *Orbital: The Electronic Journal of Chemistry*, 9 (3). <http://dx.doi.org/10.17807/orbital.v0i0.894>
- James, M. J., Gibson, R. A. & Cleland, L. G. (2000) Dietary polyunsaturated fatty acids and inflammatory mediator production. *American Journal of Clinical Nutrition*, 71, 1(Suppl), 343S–348S. <http://doi: 10.1093/ajcn/71.1.343s>
- Kerdiles, O., Lay, S. & Calon, F. (2017). Omega-3 polyunsaturated fatty acids and brain health: Preclinical evidence for the prevention of neurodegenerative diseases. *Trends in Food Science and Technology*, 69, 203–213. <https://doi.org/10.1016/j.tifs.2017.09.003>
- Menezes, M. E. S., Lira, G. M. O., Mena, C. M. B. & Sant'ana, A. E. G. (2009). Nutritional value of fish off the coast of Alagoas, Brazil. *Revista Instituto Adolfo Lutz*, 68 (1), 21–28. http://periodicos.ses.sp.bvs.br/scielo.php?script=sci_abstract&pid=S0073-98552009000100003&lng=pt&nrm=iso&tlang=pt
- Mesquita, T. R., Souza, A. A., Constantino, E., Pelóglia, N. C. C., Posso, I. P. & Pires O. C. (2011). Anti-inflammatory effect of dietary supplementation with omega-3 fatty acids in rats. *Revista Dor*, 12 (4), 337–341. <https://dx.doi.org/10.1590/S1806-00132011000400010>
- Morais, S. M., Silva, K. A., Araújo, H., Vieira, I. G. P., Alves, D. R., Fontenelle, R. O. S. & Silva, A. M. S. (2017). Anacardic Acid Constituents from Cashew Nut Shell Liquid: NMR Characterization and the Effect of Unsaturation on Its Biological Activities. *Pharmaceuticals*, 10 (31). <http://doi:10.3390/ph10010031>
- Ozogul, Y. & Ozogul, F. (2007). Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black Seas. *Food Chemistry*, 100 (4), 1634–1638. <https://doi: 10.1016/j.foodchem.2005.11.047>
- Ozogul, Y., Ozogul, F. & Alagoz S. (2007). Fatty acids profiles and fat contents of commercially important seawater and freshwater fish species of Turkey: A comparative study. *Food Chemistry*, 103, 217–223. <http://doi:10.1016/j.foodchem.2006.08.009>
- Penfield, M. P. & Campbell, A. M. (1990). *Experimental Food Science*. (3th ed.) Academic Press.
- Penido, A. B., Morais, S. M., Ribeiro, A. B., Alves, D. R., Rodrigues, A. L. M., Santos, L. H. & Menezes, J. E. S. A. (2017). Medicinal Plants from Northeastern Brazil against Alzheimer's Disease. *Evidence-Based Complementary and Alternative Medicine*, 1753673. <https://doi.org/10.1155/2017/1753673>
- Pereira, A. S., Shitsuka, D. M., Parreira, F. J., & Shitsuka, R. (2018). Metodologia da pesquisa científica. [e-book]. Santa Maria. UAB/ NTE/ UFSM. https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf?sequence=1
- Prato, E. & Biandolino, F. (2012). Total lipid content and fatty acid composition of commercially important fish species from the Mediterranean, Mar Grande Sea. *Food Chemistry*, 131 (4), 1233–1239. <https://doi.org/10.1016/j.foodchem.2014.03055>
- Rhee, I. K., Meent, M. V. D., Ingkaninan, K. & Verpoorte, R. (2001). Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. *Journal of Chromatography*, 915 (1-2), 217–23. [https://doi:10.1016/s0021-9673\(01\)00624-0](https://doi:10.1016/s0021-9673(01)00624-0)
- Santos, R. C., Melo Filho, A. A., Chagas, E. A., Takahashi, J. A., Ferraz, V. P., Costa, A. K. P., Melo, A. C. G. R., Montero, I. F. & Ribeiro P. R. E. (2015). Fatty acid profile and bioactivity from *Annona hypoglauca* seeds oil. *African Journal of Biotechnology*, 14 (30). <https://dx.doi.org/10.5897/AJB2015.14714>
- Santos, T. C., Gomes, T. M., Pinto, B. A. S., Camara, A. L. & Paes, A. M. A. (2018). Naturally Occurring Acetylcholinesterase Inhibitors and Their Potential Use for Alzheimer's Disease Therapy. *Frontiers in Pharmacology*, 18 (9), 1192. <https://dx.doi.org/10.3389/fphar.2018.01192>
- Song, C., Shieh, C., Wu, Y., Kalueff, A., Gaikwad, S. & Su, K. (2016). The role of omega-3 polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids in the treatment of major depression and Alzheimer's disease: Acting separately or synergistically? *Progress in Lipid Research*, 62, 41–54. <https://doi: 10.1016/j.plipres.2015.12.003>
- Trevisan, M. T. S., Macedo, F. V. V., Van Den Meent, M., Rhee, I. K. & Verpoorte, R.. (2003) Seleção de plantas com atividade anticolinesterase para tratamento da doença de Alzheimer. *Química Nova*, 26 (3), 301-4. <https://dx.doi.org/10.1590/S0100-40422003000300002>
- Vedin, I., Cederholm, T., Freund-Levi, Y., Basun, H., Garlind, A., Irving, G. F., Eriksdotter-Jönsson, M., Wahlund, L. O., Dahlman, I. & Palmblad, J. (2012). Effects of DHA-rich n-3 fatty acid supplementation on gene expression in blood mononuclear leukocytes: the omegaD study. *Plos One*, 7(4), e35425. <https://doi: 10.1371/journal.pone.0035425>
- Vinutha, B., Prashanth, D., Salma, K., Sreeja, S. L., Pratiti, D., Padmaja, R., Radhika, S., Amit, A., Venkateshwarlu, K. & Deepak, M. (2007). Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. *Journal of Ethnopharmacology*, 109 (2), 359–363. <https://doi:10.1016/j.jep.2006.06.014>
- Wu, S., Ding, Y., Wu, F., Li, R., Hou, J. & Mao, P. (2015) Omega-3 fatty acids intake and risks of dementia and Alzheimer's disease: A meta-analysis. *Neuroscience & Biobehavioral Reviews*, 48, 1–9. <https://doi:10.1016/j.neubiorev.2014.11.008>