

Fatty Acid and Cholesterol Concentrations in Usually Consumed Fish in Brazil

Carlos Scherr¹, Ana Carolina Moron Gagliardi², Marcio Hiroshi Miname², Raul Dias Santos²

Instituto do Coração e do Diabetes¹, Rio de Janeiro, RJ; Unidade Clínica de Lipídeos - Instituto do Coração (InCor) - Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo², São Paulo, SP - Brazil

Abstract

Background: Several studies have demonstrated clinical benefits of fish consumption for the cardiovascular system. These effects are attributed to the increased amounts of polyunsaturated fatty acids in these foods. However, the concentrations of fatty acids may vary according to region.

Objective: The goal of this study was to determine the amount of cholesterol and fatty acids in 10 Brazilian fishes and in a non-native farmed salmon usually consumed in Brazil.

Methods: The concentrations of cholesterol and fatty acids, especially omega-3, were determined in grilled fishes. Each fish sample was divided in 3 sub-samples (chops) and each one was extracted from the fish to minimize possible differences in muscle and fat contents.

Results: The largest cholesterol amount was found in white grouper (107.6 mg/100 g of fish) and the smallest in badejo (70 mg/100 g). Omega-3 amount varied from 0.01 g/100 g in badejo to 0.900 g/100 g in weakfish. Saturated fat varied from 0.687 g/100 g in seabass to 4.530 g/100 g in filhote. The salmon had the greatest concentration of polyunsaturated fats (3.29 g/100 g) and the highest content of monounsaturated was found in pescadinha (5.98 g/100 g).

Whiting and boyfriend had the best omega-6/omega 3 ratios respectively 2.22 and 1.19, however these species showed very little amounts of omega-3.

Conclusion: All studied Brazilian fishes and imported salmon have low amounts of saturated fat and most of them also have low amounts of omega-3. (Arq Bras Cardiol. 2015; 104(2):152-158)

Keywords: Fatty Acids; Cholesterol; Fishes; Coronary Artery Disease / prevention & control; Dyslipidemias.

Introduction

Several studies have reported that cardiovascular diseases are associated with lifestyles, particularly food habits¹. In 2002, Hu and Willet² published a broader review on those studies and concluded that the following three dietary strategies were effective in preventing coronary artery disease: 1) replacing saturated fats or trans fats with poly/monounsaturated fat; 2) increasing the omega-3 fatty acid consumption; 3) and consuming more fruits, vegetables, nuts, whole grains, and avoiding refined carbohydrates.

There are extensive evidence associating the increase in omega-3 consumption with lower risks of cardiovascular diseases³⁻⁵. From the epidemiological point of view, increased fish consumption is associated with lower mortality and

cardiovascular morbidity. In addition, numerous studies have showed that fish consumption has positive effects on the lipoprotein metabolism, clotting and platelet function, endothelial function, and arterial stiffness⁶.

Finally, it is important to point out that all fishes do not contain similar omega-3 concentration. For example, fishes from icy and deep waters exhibit better protective effects because of the phytoplankton diversity they feed on. Therefore, protective effects should not be generalized for all species or on the way they are prepared or cooked^{7,8}.

The present study aimed to determine the composition of fatty acids and cholesterol in the most consumed fishes in Brazil. Fishes from all over the country as well as the non-native farmed salmon, which is mostly imported from Chile, were analyzed.

Material and Methods

Fishes were obtained from local markets in various regions of the country. Fish processing was performed at the Meat Technology Center (Centro de Tecnologia de Carnes) of the Institute of Food Technology (Instituto de Tecnologia de Alimentos). A standard protocol was used for this type of study in which fishes were cleaned and processed to provide

Mailing Address: Ana Carolina Moron Gagliardi Miguel •
Rua Bernardo Ferraz de Almeida, 187 apt.º 11, Jardim Faculdade.
Postal Code 18030-290, Sorocaba, São Paulo - Brazil.
E-mail: anacarolina.moron@gmail.com
Manuscript received December 27, 2013, revised August 21, 2014,
accepted August 25, 2014.

approximately 1 kg/preparation. Then, the preparations were homogenized in a cutter and vacuum packed for a single serving.

Three fishes of the salmon and namorado species, respectively, were used, and a sample from each fish was cut, totaling to three samples per species. Each sample was divided in three subsamples (chops), and a section from each was cut from the beginning, middle, and end of the fish. Parts such as the head and tail that are usually not consumed by people were discarded and the remaining parts of the sample were cut from the one-third initial, middle, and end of the fish. This procedure intended to minimize problems regarding possible differences between fats and muscles, which could influence the final analysis results. For the other species, 1 kg of sample, which consisted of several fishes belonging to the same species for each type of preparation, was used.

Preparation of samples

Tissue compositions for cholesterol and fatty acids from 10 fishes were analyzed; of these, nine are found in Brazil and one is mostly imported from Chile (non-native farmed salmon). Of the fishes found in Brazil, three inhabit freshwater (filhote, trout, and sea bass), three inhabit salty waters of the Brazilian coast (namorado, weakfish, and sardines), and three inhabit the offshore area (whiting, robalo, and white grouper). These fishes were chosen because of their high consumption and distribution offshore (three), in the coast (three) and in freshwater (Belém, Manaus, and São Paulo).

All fishes were grilled (standard preparation), and 1 kg samples were separated without adding any other ingredient. The grill was heated and fishes were placed on it for approximately 20 min until one side was golden brown. They were turned only once with a total time of approximately 40 min on the grill.

After the grilling process, the composition of fats was analyzed by gas chromatography, consisting of the separation and subsequent quantification of fatty acids. Specific methods were used to evaluate total lipids⁹⁻¹¹, cholesterol¹²⁻¹⁴, and fatty acids¹³. As a standard, cholesterol was quantified in mg/100 g of fish and fatty acids in g/100 g of fish. Then, the ratio of the fatty acids omega-6/omega-3 was calculated.

The results were evaluated on the basis of the recommendation by the National Agency of Sanitary Surveillance (Anvisa, Agência Nacional de Vigilância Sanitária), which suggest a 2,000 kcal diet a day, including a lipid intake of up to 55 g/day; however, it should include < 22 g/day of saturated fatty acids and up to 300 mg/day of cholesterol¹⁵.

Statistical analysis

Data are showed as mean \pm standard deviation. The normality of the data was tested by the Kolmogorov–Smirnov test. ANOVA test and post-hoc Bonferroni correction were used to compare the quantification of total lipids, fatty acids, and cholesterol among the 10 different types of grilled fishes for the seven variable studied. Results were considered significant at 5% significance level.

Results

Table 1 shows the total lipid composition in fishes assessed. Table 2 shows the differences that were statistically significant among the analyzed fishes. Significant differences in the concentrations of cholesterol and fatty acids in different species were observed. The average content of cholesterol ranged from 70 mg/100 g in whiting to 107.6 mg/100 g in white grouper ($P < 0.05$). Therefore, to overcome the daily recommended amounts of cholesterol, at least 428 g/day or 279 g/day of whiting or white grouper, respectively, should be consumed¹⁶. However, there was no significant difference between the species in the highest cholesterol content (white

Table 1 - Total lipids found in the fishes assessed

	Cholesterol (mg/100 g) $p < 0,01^*$	Saturated fats (g/100 g) $p < 0,01^*$	Polyunsaturated fats (g/100 g) $p < 0,01^*$	Monounsaturated fats (g/100 g) $p < 0,01^*$
Whiting	70,03 \pm 1,68	0,69 \pm 0,03	0,03 \pm 0,00	0,37 \pm 0,01
White grouper	107,61 \pm 2,91	1,56 \pm 0,02	0,44 \pm 0,12	2,50 \pm 0,05
Filhote	94,31 \pm 0,88	4,53 \pm 0,07	1,84 \pm 0,03	3,73 \pm 0,07
Namorado	73,49 \pm 0,80	0,70 \pm 0,02	0,08 \pm 0,01	0,63 \pm 0,01
Weakfish	84,90 \pm 2,34	2,13 \pm 0,07	1,26 \pm 0,09	5,98 \pm 0,25
Seabass	88,12 \pm 3,84	1,76 \pm 0,02	0,18 \pm 0,03	1,21 \pm 0,04
Robalo	73,76 \pm 2,50	0,68 \pm 0,02	0,07 \pm 0,10	0,34 \pm 0,005
Sardine	86,05 \pm 1,55	1,85 \pm 0,05	0,02 \pm 0,00	0,60 \pm 0,03
Trout	86,82 \pm 3,50	2,57 \pm 0,04	1,60 \pm 0,06	4,03 \pm 0,07
Salmon	93,33 \pm 18,42	2,57 \pm 0,66	3,11 \pm 0,72	2,41 \pm 0,71

* ANOVA: difference among the various fishes assessed.

Table 2 – Breakdown of the differences found in the post-hoc Bonferroni correction among fishes assessed

Comparison among fishes/type of fat assessed	Cholesterol	Saturated fats	Polyunsaturated fats	Monounsaturated fats
Whiting	White grouper, filhote	White grouper, filhote, weakfish, seabass, trout, salmon	Weakfish, trout, salmon	White grouper, filhote, namorado, weakfish, seabass, sardine, trout, salmon
White grouper	Whiting, namorado, robalo, sardine, trout	Whiting, filhote, namorado, robalo, trout, salmon	Weakfish, trout, salmon	Whiting, filhote, namorado, weakfish, seabass, robalo, sardine, trout
Filhote	Whiting, namorado, robalo	Whiting, white grouper, namorado, weakfish, seabass, robalo, sardine, trout, salmon	Whiting, white grouper, namorado, seabass, robalo, sardine, salmon	Whiting, white grouper, namorado, weakfish, seabass, robalo, sardine, salmon
Namorado	White grouper, filhote, trout	White grouper, filhote, weakfish, seabass, sardine, trout, salmon	Filhote, weakfish, trout, salmon	White grouper, filhote, weakfish, trout, salmon
Weakfish	White grouper	Whiting, filhote, namorado, robalo	Namorado, seabass, robalo, sardine, salmon	Whiting, white grouper, filhote, namorado, seabass, robalo, sardine, trout, salmon
Seabass	White grouper	Filhote, namorado, robalo, trout, salmon	Filhote, weakfish, trout, salmon	Whiting, white grouper, filhote, weakfish, robalo, trout, salmon
Robalo	White grouper, filhote, salmon	White grouper, filhote, weakfish, seabass, sardine, trout, salmon	Filhote, weakfish, trout, salmon	White grouper, filhote, weakfish, seabass, trout, salmon
Sardine	White grouper	Filhote, namorado, robalo, trout, salmon	Filhote, weakfish, trout, salmon	White grouper, filhote, weakfish, trout, salmon
Trout	White grouper	Whiting, white grouper, filhote, namorado, seabass, robalo, sardine, salmon	Whiting, white grouper, namorado, seabass, robalo, sardine, salmon	Whiting, filhote, namorado, weakfish, seabass, robalo, sardine, salmon

Lines show statistical differences (*p* value < 0.05).

grouper versus filhote, *p* = 0.776; white grouper vs. salmon, *p* = 0.511).

Saturated fat analysis revealed that the content of saturated fats were not similar among fishes, ranging from 0.68 g/100 g in sea bass to 4.53 g/100 g in filhote. Therefore, to exceed the recommended saturated fat levels, it would be necessary to consume 3,200 g/day of sea bass or 486 g/day of filhote¹⁶.

Unsaturated fatty acid analysis showed that salmon, filhote, and trout (*p* < 0.01 vs. other fish species) are the species with the highest concentration of polyunsaturated fats, whereas weakfish, trout, and filhote have the most significant concentration of monounsaturated fats (*p* < 0.01 vs. other fish species).

Table 3 shows the concentrations of omega-6 and omega-3 and their ratio. Variables were statistically different among fishes (*p* < 0.01). Table 4 shows the differences that were statistically significant among the fishes analyzed.

Most fishes had low omega-3 concentrations. For example, the omega-3 concentration in whiting was 0.009 g/100 g, requiring an individual to consume at least 20 kg/day of whiting to achieve the recommended amount of omega-3 (2 g/day)¹⁶. On the other hand, weakfish (0.9 g/100 g), salmon

(0.79 g/100 g), and filhote (0.38 g/100 g) had the highest omega-3 concentration. Salmon is best known as a source of omega-3.

According to other studies, the ideal ratio between omega-6 and omega-3 is 1/1 or 2/1¹⁷. Thus, whiting (2.22) and namorado (1.19) had the best ratios; there is no statistic difference between them (*p* = 0.228). The worst ratios were found in trout (9.03) and sea bass (5.25), with a statistic difference of *p* < 0.05. However, despite their good ratios, whiting and namorado had very low omega-3 concentrations.

Discussion

In this study, we quantified fats found in the most consumed fishes in Brazil. Analyses were done once fishes were grilled, and our data showed important variations among species. Our results show that all fishes had low saturated fat levels; however, they also had small omega-3 concentrations. Differences in cholesterol concentrations among fishes were observed as well.

Cholesterol intake, particularly from saturated fats, is associated with increasing LDL-cholesterol (LDL-C) concentrations in the blood¹⁶, which is the opposite when poly/monounsaturated fats are consumed. Both cholesterol

and dietary fats exert effects upon cholesterolemia by modulating the expression of LDL receptors in the liver¹⁶.

Dietary cholesterol intake can change blood LDL-C concentrations. It is not recommended to consume > 200 mg/day of cholesterol in a healthy diet¹⁶. Therefore, white grouper should be consumed as part of a primary cardiovascular prevention and should not exceed 186 g/day in patients who are undergoing secondary cardiovascular prevention. However, it is important to emphasize that cholesterolemia is determined mostly because of saturated fatty acid consumption instead of dietary cholesterol¹⁶. Therefore, some questions remain regarding whether fishes consumption with the highest content of cholesterol has deleterious effects on the cardiovascular health.

Brazilian guidelines for fat consumption and cardiovascular health¹⁶ states that more mono/poly-unsaturated fats (20% and 10%, respectively) should be consumed, with reduced amounts of saturated fat (up to 10% of total caloric intake)¹⁶. Based on this guideline, filhote is the species that better matches these recommendations.

Regarding omega-3, weakfish presented the highest tissue concentrations, making it a good source of omega-3, particularly if at least 222 g/day of this fish is consumed. Second comes the salmon, and a consumption of 253 g/day is recommended to achieve the recommendations of 2 g/day of omega-3 to prevent cardiovascular diseases. On the other hand, whiting had such low omega-3 concentrations that at least 20 kg of this fish would have to be consumed to achieve levels that are usually associated with the reduction of cardiovascular risks. This amount is unfeasible for human consumption on a daily basis; the same applies to sea bass and namorado.

Epidemiological and intervention studies associate fish consumption with better lipid profiles and reduced risk of cardiovascular disease¹⁸⁻²³. This could be explained by the fact that fish have the lowest saturated fat and

cholesterol concentrations and greater polyunsaturated fat concentrations. Furthermore, there are possible functional effects of omega-3 sourced from deep sea fishes, such as antiplatelet and antiarrhythmic actions^{6,16}.

The Chicago Western Electric study, which assessed 1,822 men between 40–55 years old, for 30 years, showed that fish consumption was inversely associated with mortality from coronary disease¹⁸. Similar data were found in the cohort of Zutphen in The Netherlands, where 852 middle-aged men were studied for 20 years. The consumption of at least 30 g/day of freshwater fish, regardless the species, was associated with 50% reduction in mortality from coronary heart disease¹⁹.

Positive results were also discussed in a major review by Mozaffarian (2011), regarding sudden cardiac death and higher fish consumption. This review was focused on polyunsaturated fat intake, particularly omega-3²⁰.

The Nurse's Health Study, performed in the United States with 85,000 women, showed that the consumption of two to four servings of fish per week reduced one-third risks of heart diseases. There were benefits in even those who consumed fish only one to three times a month. As a result of this research, the American Heart Association began recommending the consumption of two servings of fish per week²¹.

Even in lower-risk groups, such as young women, studies have found beneficial effect on reducing cardiovascular risks in those who consume more fish compared with those who eat little or do not consume fish at all²².

Evidence of fish consumption benefits also exist in secondary cardiovascular prevention, as shown in the Diet and Reinfarction Trial. They studied 2,000 men in secondary prevention for myocardial infarction for 2 years. Randomized into four groups, those who consumed more fish had 29% lower mortality compared with the controls and had fewer fatal infarcts²³.

Table 3 – Concentration of omega-3 and omega-6 fatty acids and their ratio in fishes assessed

	Omega-6 (g/100 g) p < 0,01*	Omega-3 (g/100 g) p < 0,01*	Ratio omega 6/3 p < 0,01*
Whiting	0,02 ± 0,00	0,009 ± 0,00	2,22 ± 0,00
White grouper	0,16 ± 0,02	0,27 ± 0,10	0,64 ± 0,20
Filhote	1,46 ± 0,01	0,38 ± 0,01	3,85 ± 0,13
Namorado	0,04 ± 0,005	0,04 ± 0,01	1,19 ± 0,17
Weakfish	0,36 ± 0,02	0,90 ± 0,11	0,40 ± 0,07
Seabass	0,15 ± 0,02	0,03 ± 0,01	5,25 ± 1,14
Robalo	0,016 ± 0,005	0,01 ± 0,00	0,18 ± 0,06
Sardine	0,02 ± 0,00	0,09 ± 0,00	0,22 ± 0,00
Trout	1,44 ± 0,05	0,16 ± 0,01	9,03 ± 0,43
Salmon	0,29 ± 0,07	0,79 ± 0,19	0,36 ± 0,016

*ANOVA: difference among the various fishes assessed.

Table 4 – Breakdown of the differences found in the post hoc analysis with Bonferroni correction among fishes assessed

	Omega-6 p < 0,01*	Omega-3 p < 0,01*	Ratio omega 6/3 p < 0,01*
Whiting	White grouper, filhote, weakfish, seabass, trout, salmon	White grouper, filhote, weakfish, salmon	White grouper, filhote, weakfish, seabass, robalo, sardine, trout, salmon
White grouper	Whiting, filhote, namorado, weakfish, robalo, sardine, trout, salmon	Whiting, weakfish, seabass, salmon	Whiting, filhote, seabass, trout
Filhote	Whiting, white grouper, namorado, weakfish, seabass, robalo, sardine, trout	Whiting, namorado, weakfish, seabass, robalo, sardine, salmon	Whiting, white grouper, namorado, weakfish, seabass, robalo, sardine, trout, salmon
Namorado	White grouper, filhote, weakfish, seabass, trout, salmon	Filhote, weakfish, salmon	Filhote, seabass, trout
Weakfish	Whiting, white grouper, filhote, namorado, seabass, robalo, sardine, trout	Whiting, white grouper, filhote, namorado, seabass, robalo, sardine, trout	Whiting, filhote, seabass, trout
Seabass	Whiting, filhote, namorado, weakfish, robalo, sardine, trout, salmon	White grouper, filhote, weakfish, salmon	Whiting, white grouper, filhote, namorado, weakfish, robalo, sardine, trout, salmon
Robalo	White grouper, filhote, weakfish, seabass, trout, salmon	Filhote, weakfish, salmon	Whiting, filhote, seabass, trout
Sardine	White grouper, filhote, weakfish, seabass, trout, salmon	Filhote, weakfish, salmon	Whiting, filhote, seabass, trout
Trout	Whiting, white grouper, namorado, weakfish, seabass, robalo, sardine, salmon	Weakfish, salmon	White grouper, filhote, namorado, weakfish, robalo, sardine, trout, salmon
Salmon	Whiting, white grouper, filhote, namorado, seabass, robalo, sardine, trout, salmon	Whiting, white grouper, filhote, namorado, weakfish, seabass, robalo, sardine, trout	Whiting, filhote, seabass, trout

Lines show statistical differences (p value < 0.05).

Fish intake has been recommended as part of a Mediterranean-style diet, and its benefits in cardiovascular prevention was shown in the Italian cohort GISSI-Prevenzione. This study comprised 172 centers that followed up with 11,323 men and women for approximately 6 and a half years; all patients presented a history of myocardial infarction. They were encouraged to increase fish, fruits and raw or cooked vegetables, and olive oil consumptions. Individuals who followed the recommendations had lower risk of cardiovascular events²⁴. This study also provided patients with supplemental eicosapentaenoic acid and docosahexaenoic acid at an average dosage of 850 mg/day. Omega-3 consumption from deep sea fishes was associated with a significant reduction in deaths from coronary heart diseases (30% reduction) and sudden cardiac death (45% reduction).

A review spanning seven cohorts and one control case study also showed that the amount of omega-3 intake was correlated with greater benefits for those who consumed > 250 mg/day of fish compared with those who consumed less^{25,26}.

Therefore, not only is fish consumption beneficial in itself but their omega-3 concentration can have positive effect. This, in turn, is related to the species and its origin. In our study, all species presented low omega-3 concentrations, which may be related to the type of food consumed by them²⁷ and incorporated into their fatty tissue.

Altogether, our study showed low omega-3 concentrations in most Brazilian fishes assessed the differences in fats among the species. Therefore, questions remain whether all fishes bring the same beneficial effects or only specific species, such as the ones that live in cold water²⁷.

Despite our findings, we believe that substitution of foods such as meats and dairy products, which have the highest saturated fat content, with fish is recommended as part of a diet to prevent cardiovascular diseases.

Limitations

In this study, we assessed only some types of fish and only one preparation method. It is possible that the preparation method may influence the chemical composition of cholesterol and fatty acids of fishes. In addition, there may be variations in the composition of fats in fishes of the same species or in those captured from different locations along the Brazilian coast due to the variation in the phytoplankton^{28,29}.

Conclusion

Fishes analyzed in our study presented low saturated fat content but some may have high cholesterol concentrations, as is the case of white grouper. Omega-3 concentrations were low in most fishes analyzed.

Author contributions

Conception and design of the research, Acquisition of data and Obtaining financing: Scherr C; Analysis and interpretation of the data, Statistical analysis and Critical revision of the manuscript for intellectual content: Scherr C, Gagliardi ACM, Miname MH, Santos RD; Writing of the manuscript: Scherr C, Gagliardi ACM, Santos RD.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by Instituto Nacional de Metrologia e Estatística.

Study Association

This study is not associated with any thesis or dissertation work.

References

- Scherr C. Influência dos hábitos de vida do perfil lipídico dos pacientes coronariopatas. [Dissertação]. Rio de Janeiro: Universidade Federal Fluminense / Best Point Editora; 2004.
- Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. *JAMA*. 2002;288(20):2569-78.
- Mozaffarian D, Lemaitre RN, King LB, Song X, Huang H, Sacks FM, et al. Plasma phospholipid long-chain ω -3 fatty acids and total and cause-specific mortality in older adults: a cohort study. *Ann Intern Med*. 2013;158(7):515-25.
- Yamagishi K, Nettleton JA, Folsom AR; ARIC Study Investigators. Plasma fatty acid composition and incident heart failure in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J*. 2008;156(5):965-74.
- Mancardi D, Tullio F, Crisafulli A, Rastaldo R, Folino A, Penna C, et al. Omega 3 has a beneficial effect on ischemia/reperfusion injury, but cannot reverse the effect of stressful forced exercise. *Nutr Metab Cardiovasc Dis*. 2009;19(1):20-6.
- Vrablík M, Prusíková M, Snejdlrová M, Zlatohlávek L. Omega-3 fatty acids and cardiovascular disease risk: do we understand the relationship? *Physiol Res*. 2009;58 Suppl 1:S19-26.
- Gómez F, Souissi S. The impact of the 2003 summer heat wave and the 2005 late cold wave on the phytoplankton in the northeastern English Channel. *C R Biol*. 2008;331(9):678-85.
- Paz B, Vázquez JA, Riobó P, Franco JM. Study of the effect of temperature, irradiance and salinity on growth and yessotoxin production by the dinoflagellate *Protoceratium reticulatum* in culture by using a kinetic and factorial approach. *Mar Environ Res*. 2006;62(4):286-300.
- Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*. 1957;226(1):497-509.
- Gerber A, Van Gullik D. Normas analíticas do Instituto Adolfo Lutz. 3ª. ed. São Paulo: Instituto Adolfo Lutz; 1985. p. 270.
- Hartman L, Lago RCA. Rapid preparation of fatty acid methyl esters from lipids. *Lab Pract*. 1973;22(6):475-6.
- Bragagnolo N, Rodriguez-Amaya DB. Avaliação comparativa de três métodos para determinação de colesterol em gema de ovo. *Arq Biol Tecnol*. 1993;36(2):237-51.
- Schmarr H, Gross HB, Shibamoto T. Analysis of polar cholesterol oxidation products: evaluation of a new method involving transesterification, solid phase extraction, and gas chromatography. *J Agric Food Chem*. 1996;44:512-7.
- Firestone, D. Official methods and recommended practices of the American Oil Chemists Society. 5th ed. Rev. Champaign: AOCS. 2007. Met. Ce 1e-91, Ce 1f-96, Ce 1-62. Current through Revision 1; 2008.
- Ministério da Saúde. Anvisa. Resolução RDC n. 360, de 23 de dezembro de 2003: aprova regulamento técnico sobre rotulagem nutricional de alimentos embalados, tornando obrigatória a rotulagem nutricional. D.O.U. Diário Oficial da União; 26 de dezembro de 2003.
- Santos RD, Gagliardi ACM, Xavier HT, Magnoni CD, Cassani R, Lottenberg AM, et al. Sociedade Brasileira de Cardiologia. I Diretriz sobre o consumo de gorduras e saúde cardiovascular. *Arq Bras Cardiol*. 2013;100(1 Supl.3):1-40.
- Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother*. 2002;56(8):365-79.
- Daviglus ML, Stamler J, Orenca AJ, Dyer AR, Liu K, Greenland P, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. *N Engl J Med*. 1997;336(15):1046-53.
- Kromhout D, Bosschieter EB, De Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med*. 1985;312(19):1205-9.
- Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol*. 2011;58(20):2047-67.
- Baer HJ, Glynn RJ, Hu FB, Hankinson SE, Willett WC, Colditz GA, et al. Risk factors for mortality in the nurses' health study: a competing risks analysis. *Am J Epidemiol*. 2011;173(3):319-29.
- Strøm M, Halldorsson TI, Mortensen EL, Torp-Pedersen C, Olsen SF. Fish, n-3 fatty acids, and cardiovascular diseases in women of reproductive age: a prospective study in a large national cohort. *Hypertension*. 2012;59(1):36-43.
- Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet*. 1989;2(8666):757-61.
- Barzi F, Woodward M, Marfisi RM, Tavazi L, Valagussa F, Marchi R; GISSI-Prevenzione Investigators. Mediterranean diet and all-causes mortality after myocardial infarction: results from GISSI-Prevenzione trial. *Eur J Clin Nutr*. 2003;57(4):604-11. Erratum in *Eur J Clin Nutr*. 2003;57(8):1034.
- Musa-Veloso K, Binns MA, Kocenas A, Chung C, Rice H, Oppedal-Olsen H, et al. Impact of low v. moderate intakes of long-chain n-3 fatty acids on risk of coronary heart disease. *Br J Nutr*. 2011;106(8):1129-41.
- Streppel MT, Ocké MC, Boshuizen HC, Kok FJ, Kromhout D. Long-term fish consumption and n-3 fatty acid intake in relation to (sudden) coronary heart disease death: the Zutphen study. *Eur Heart J*. 2008;29(16):2024-30.
- Hunter DJ, Kazda I, Chockalingam A, Fodor JG. Fish consumption and cardiovascular mortality in Canada: an inter-regional comparison. *Am J Prev Med*. 1988;4(1):5-10.
- Midtbø LK, Ibrahim MM, Myrmet LS, Aune UL, Alvheim AR, Liland NS, et al. Intake of farmed Atlantic salmon fed soybean oil increases insulin resistance and hepatic lipid accumulation in mice. *PLoS One*. 2013;8(1):e53094.
- Nichols PD, Glencross B, Petrie JR, Singh SP. Readily available sources of long-chain omega-3 oils: is farmed Australian seafood a better source of the good oil than wild-caught seafood? *Nutrients*. 2014;6(3):1063-79.

