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# The influence of season on the lipid profiles of five commercially important species of Brazilian fish

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## Abstract

This study aimed at determining the influence of season (summer and winter) the total lipid, fatty acid and cholesterol contents of five popular fish species: the sardine, Sardinella spp., the croaker Micropogonias furnieri (marine species), the curimbatá Prochilodus spp., the tilápia, Oreochromis spp. (fresh water species), and the seabob shrimp, Xiphopenaeus kroyeri. Total lipid analysis was performed by the dry column; fatty acids were determined by gas chromatography and cholesterol was determined by a colorimetric method. Statistical treatment of results showed that sardines collected during winter had the highest lipid contents (10.62). The sardine, the croaker, the tilápia and the curimbatá were not influenced by seasonality in terms of their total saturated and unsaturated acid contents. The highest contents of eicosapentanoic acid (3.02 and 1.87%) and docosahexaenoic acid (10.1 and 11.3%) were found in the sardine. The shrimp presented the highest cholesterol contents (165 mg/100 g in summer and 165 mg/100 g in winter).

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Keywords: Lipid profile; Fish and cholesterol

# 1. Introduction

Recently, countless studies have been carried out with a view to improving the understanding of disease pathogenesis and its association with food, with increasing emphasis on the role of lipids. There is much evidence that the fatty acid content of a diet modulates the fatty acid profile of immune cells. Therefore, this may be an effective way to regulate the functionality of normal cells through nutrition. In fact, many studies have concluded that diets in which unsaturated fatty acids replace the saturated ones are associated with low incidence of coronary diseases [\(Fuentes, 1998; Mensink](#page-3-0) [& Katan, 1990](#page-3-0)). This stems from the fact that polyunsaturated fatty acids of the o3 family, especially eicosapentanoic acid, influence the production of prothrombotic prostaglandin and thromboxane or are converted into anti-thrombotic prostaglandins ([Good](#page-3-0)[night, Harris, Connor, & Allingworth, 1982](#page-3-0)). [Mattson,](#page-4-0) [Erickson, and Kligman \(1972\)](#page-4-0) revealed the existence of a linear relationship between dietary cholesterol and blood cholesterol. Each 100 mg of cholesterol per 1000 kcal consumed resulted in an increase of 12 mg of cholesterol per 100 ml of blood. A normal Western person consumes between 600 and 1000 mg of cholesterol a day on average. According to the [World Health Organiza](#page-4-0)[tion \(1982\),](#page-4-0) the maximum recommended intake is 300 mg/day. Taking these facts into account, all attempts to reduce the risk of the earlier-mentioned diseases, especially cardiovascular diseases, emphasize the importance of an increased consumption of fish or fish products, which are rich in polyunsaturated fatty acids of the o3 family and poor in polyunsaturated fatty acids of the o6 family [\(Burr, 1989; Sargent, 1997](#page-3-0)). In Brazil, however, in spite of there being a great variety of aquatic species, the contribution of fish to the diet is small; it is thus very important to change the habits of the population. In view of these facts, it seemed necessary to carry out a study on the nutritional value and lipid profile of highly consumed fishes in two different seasons. The object of this work is therefore to characterize five fish species in terms of their lipid, fatty acid and cholesterol contents in two different seasons of the year.

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# <span id="page-1-0"></span>2. Materials and methods

## 2.1. Collection of samples

Five fish species were analysed: the sardine, Sardinella spp., the croaker Micropogonias furnieri, seabob shrimp, Xiphopenaeus kroyeri (marine species), the curimbata´ Prochilodus spp. and the tilápia, Oreochromis spp. (fresh water species). The selection criterion was the volume of sales for the species according to the 1997 yearly Bulletin of the General Warehouse Company of the State of São Paulo ([CEAGESP, 1997](#page-3-0)), in the City of São Paulo, where the samples were obtained through donation. The seasons chosen for analysis were summer and winter, and the samples were collected in one month of each season during 1999 (Table 1).

# 2.2. Preparation of the fish samples

The samples comprised five specimens of sardine, two of croaker, two of tilápia, one of curimbatá and approximately 300 g of seabob shrimps. Four lots of each species were analysed during summer and during winter as well, and each lot was analysed in triplicate (Table 2). In the case of the tilápia, three lots were analysed during summer due to the impossibility of obtaining

Table 1

Biometric data of the species under analysis

Date	<b>Species</b>									
	Sardine		Croaker		Tilápia		Curimatá		Shrimp	
	$g^*$	$cm^*$ g <sup>*</sup>		$cm^* g^*$		$cm^*$ $g^*$		$cm^*$ $g^*$		
99/03/03 64.1			17.3 1080.0 46.6 513.2 31.5 1852.3 50.0 355.0							
99/03/09 98.4		19.4					714.0 41.0 772.3 35.0 1428.7 38.0 585.4			
99/03/16 85.6		19.1	469.6 30.5 586.4 26.3 1806.0 45.0 299.8							
99/03/23 58.8		14.6	533.9 32.0				w.s. w.s. 1662.5 43.0 355.4			
99/07/06 55.6		17.4					671.8 38.0 279.5 21.0 1434.5 49.0 436.3			
99/07/14 48.6		16.0					596.8 37.0 308.4 23.0 1813.8 47.0 331.0			
99/07/20 60.4			15.5 561.6 34.0 521.0 26.0 1432.6 40.0 453.9							
99/07/27 69.1		16.0					701.0 36.0 425.6 24.2 1637.9 43.0 574.6			

\*Average of four lots analysed in triplicate (g weight and cm size).

Table 2

Lipid values determined in summer and winter for the species analyzed

Species	Lipids $(\%$ wet weight basis)			
	Summer	Winter		
Sardine	4.00a	10.62a		
Croaker	0.60a	3.29 <sub>b</sub>		
Tilápia	1.92a	133a		
Curimbatá	9.70a	6.67a		
Shrimp	0.94a	1.16b		

Average of four lots analysed in triplicate. Averages followed by the same letter (columns) show no statistical differences  $(P<0.01)$ according to the MSD (minimum significant differences) test.

samples on the date set for the analysis. Each specimen of fish, after having its biometrics data (weight and length) measured, had its head cut off and was eviscerated and filleted, all manually. As for the shrimp samples, total weight was determined in order to calculate the average weight per specimen; then, following the procedure described earlier; all specimens had their heads cut off and were shelled.

# 2.3. Fatty acid determination

#### 2.3.1. Total fat

The lipids were determined using the dry column methodology suggested by [Marmer and Maxwell](#page-4-0) [\(1981\)](#page-4-0), which offers a viable alternative to extraction using chloroform, methanol, and water. The beakers employed were previously stove-dried for 12 h at 105  $\degree$ C, after this they were cooled in a desiccator and weighed. Ten millilitres of the dry column extract were transferred to the beaker and evaporated under nitrogen. Afterwards the extract was placed in a stove at  $105^{\circ}$ C and, after 3 h, it was cooled in a desiccator and weighed on an analytical balance.

## 2.3.2. Total fatty acids (FA)

Lipids were submitted to cold saponification and methylation with  $BF_3$  in methanol ([Morrison & Smith,](#page-4-0) [1974\)](#page-4-0). The determination of FA was conducted in a GC Chrompack CP9002, split injector in a ratio 100:1, FID and capillary column of fused silica CP-SIL 88 (50 m;  $0.25$  mm and  $0.25$  µm). The C17:0 was used as internal standard and the fatty acid methyl ester mixture No. 189-19 was used for quantitative external standards; they were both from  $Sigma^{\circledR}$ . Normalizing the area and identification allowed quantification of the TFA by comparison of corrected retention times between standards and samples.

## 2.4. Cholesterol determination

#### 2.4.1. Cholesterol

The non-saponifiable lipids were analyzed through the colorimetric method of Bohac, Rhee, Cross, and Ono (1988), according to whom this is an efficient method which yields similar results to those achieved through gaseous chromatography, with the advantage of being less costly. The lipids were extracted and analysed by the technique described by [Marmer and Maxwell](#page-4-0) [\(1981\)](#page-4-0). Three millilitres of extract, taken from each sample, were evaporated under nitrogen. After being dried, the samples were saponified by the addition of 10 ml of a 12% potassium hydroxide (KOH) solution in ethanol and subjected to an 80  $\degree$ C water bath with agitation for 15 min. They were then promptly cooled through the addition of 5 ml of distilled, demineralized water followed by double extraction with 10 ml of hexane. Four-millilitre

aliquots of the hexane were taken and dried under nitrogen  $(N_2)$  flow. Six millilitres of a saturated solution of ferrous sulphate in glacial acetic acid were added, as well as 2 ml of concentrated sulfuric acid  $(H_2SO_4)$ . The samples thus treated were read with a "Coleman-295" spectrophotometer at 490 nm. The calibration curve was constructed based on 50, 100, 150, and 200  $\mu$ g solutions, subjecting the Sigma<sup>®</sup> C-8253 standard cholesterol concentrations to the saponification and colour development stages. A 10–40 mg gradient was achieved at the end of the process.

## 3. Results and discussion

Substantial differences between the seasons were observed in the cases of the croaker and the seabob shrimp. [Krzynowek and Panunzio \(1989\)](#page-4-0) studied 11 species of shrimp and found lipid values in the 0.8–1.1% range, classifying crustaceans as low-lipid foods. Similarly, [Pedraja \(1970\)](#page-4-0) stresses that the lipid content of shrimps are in fact low (0.5–0.8%) when compared with their protein contents. Researching the effect of climate on lipid content variation, [Krzynowek \(1985\)](#page-4-0) reported that the fat contents of some fish species might vary by approximately 10% according to the season of capture. [Henderson and Tocher \(1987\)](#page-3-0) listed 56 species of fresh water fish (mostly from temperate countries) whose fillets or muscles showed total lipid contents from 0.7 to 25.8% (wet basis). Also researching lipids, [Lazos, Aggelousis,](#page-4-0) [and Alexis \(1989\)](#page-4-0) studied 11 species of fresh water fish from Greece and found lipid contents in the 0.6 to 7.6% range. The contents mentioned earlier are similar to the results found for the fresh water species analysed herein [\(Table 2](#page-1-0)).

Tables 3 and 4 present the six major fatty acids (C16: 0, C 18:0, C18: 1, C18: 1,9, C20: 5 e C22: 6) accounted for 89.8%, 91.8%, 91.6%, 73.7%, and 88.7% of the total lipid contents for the sardine, the croaker, the tilápia, the curimbatá and the seabob shrimp, respectively. Among the saturated fatty acids, the palmitic acid was prevalent in all species, with the following averages: 47.5% (sardine), 38.4% (croaker), 35.9% (tilápia),  $28.9\%$  (curimbatá) and  $35.0\%$  (seabob shrimp). These values showed a 51.6% variation. Regarding fresh water fishes, some authors ([Maia,](#page-4-0) [Rodriguez-Amaya, and Amaya-Farfan, 1983; Pezzato,](#page-4-0) [1990\)](#page-4-0) have found values with 34% to 49% variations.  $\alpha$ -Linolenic acid (18:3 $\omega$ 3) was detected only in the curimbatá  $(1.53\%$  in summer and  $0.88\%$  in winter). [Bardolato et al. \(1994\)](#page-3-0) detected this acid in very small amounts and only during some seasons while studying marine fish species in different seasons of the year. Also, [Bragagnolo \(1997\),](#page-3-0) when analysing the pink spotted shrimp (Penaeus brasiliensis), found that only 0.5% of the lipids corresponded to  $\alpha$ -linolenic acid in this species; this acid was not detected in the seabob shrimp.

Eicosapentanoic acid (EPA) was the main polyenoic fatty acid found in the curimbatá. [Maia \(1992\)](#page-4-0) found linolenic acid to be the main polyenoic acid in curimbatás, followed by EPA. When the species under analysis was the tilápia, EPA prevailed. According to [Jauncey \(1982\)](#page-3-0), tilápia is a species that requires fatty acids of the o6 family—instead of o3—for its nutrition.

As regards the sum of EPA and DHA, sardines collected during winter showed the highest content, followed by curimbatás collected during summer. The lowest EPA+DHA contents in summer were found in tilápias; during winter, the lowest  $EPA + DHA$  content were found in curimbata's (Tables 3 and 4).

When n3 contents were compared, no significant differences were found among the species, although the sardine showed slightly a higher  $\omega$ 3 content (13.4%), followed by the seabob shrimp (12.9%).

Cholesterol content was higher in marine shrimp in summer and winter followed by curimbatá in summer and tila´pia had the lowest values [\(Table 5\)](#page-3-0). Cholesterol values between 50.0 and 90.0 mg/100 g were found in fish muscles ([Criner & Feeley, 1972](#page-3-0)) and in fresh water fish [\(Kinsella, Shimp, & Weihrauch, 1977\)](#page-3-0). The authors reported that cholesterol is the predominant sterol.

[Bragagnolo \(1997\)](#page-3-0) found slightly lower cholesterol values in seabob shrimps,  $134 \pm 12$  mg/100 g.

Table 3

Major fatty acids (%) of total lipids in the species analysed during summer

Fatty acids	Sardine	Croaker	Tilápia	Curimbatá	Shrimp
C14:0	4.58	0.44	0.70	3.19	
C15:0	0.11				
C16:0	46.8	38.6	35.5	27.84	35.8
C16:1	3.58	3.26	1.14	12.6	4.74
C17:0	1.51	0.42		0.26	2.30
C17:1	2.02			1.65	
C18:0	8.82	16.7	14.1	4.19	16.2
C18:1	14.5	17.7	11.5	16.7	19.50
$C18:1$ trans 9	1.47	0.90	17.0	2.11	1.70
C18:2	0.25		-		
C18:2 trans 9,12	0.23	-	8.95	2.53	
C18:3				1.53	
C20:0				6.66	
C20:2	0.16				
C20:3n3	0.30				
C20:3n6			0.51	0.20	
C20:4		3.09		0.23	
C20:5	3.02	6.74	7.46	7.47	4.26
C22:1				1.20	
C22:2	1.95	5.43		7.50	2.35
C22:6	10.1	5.90		6.01	8.23
C23:0		0.80	0.77	0.44	0.80
n <sub>3</sub>	13.4	12.6	7.46	13.5	12.5
n6	2.59	8.52	9.46	10.5	2.35
$EPA + DHA$	13.1	12.6	7.46	13.5	12.5

\*Average of four lots analysed.

<span id="page-3-0"></span>On the other hand, some authors found much higher values than the ones found in this study. Johnsoton, Ghanbari, Wheeler, and Kirk (1983) determined an average of 201 mg/100 g in P. aztecus, and [Kritchevsky,](#page-4-0) [Tepper, Dltullo, and Holmes \(1967\)](#page-4-0) an average of 200 mg/100 g in a non-identified species of shrimp.

Table 4





\*Average of four lots analysed.

Table 5





Average of four lots analysed. Averages followed by the same letter show no statistical differences  $(P<0.01)$  according to the MSD (minimum significant differences) test.

King, Childs, Dosett, Ostrander, and Monsen (1990) found that lipid and cholesterol contents were not affected by the climate in Pandalus borealis and P. jordani shrimps, although the winter lipid contents were a little higher than summer contents (1.3 and 1.2%).

# 5. Conclusions

The highest lipid contents were found in sardines analysed in summer, followed by curimbatas. The sardine, the tilápia and the shrimp were not influenced by seasonality in terms of cholesterol, and the shrimp showed the highest cholesterol contents.

The shrimp was the only species influenced by seasonality, showing higher saturated and unsaturated fatty acid contents in summer. As regards EPA+DHA contents, sardines collected during summer showed the highest value, followed by seabob shrimps collected during winter.

The sardine proved to be the species most suited to a fish-based diet due to its lipid and EPA+DHA contents.

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