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A N I N T E R N A T I O N A L J O U R N A L

Proximate Composition and Fatty Acid Content of the Mangrove Oyster *Crassostrea rhizophorae* Along the Year Seasons

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ABSTRACT

Mangrove oysters, Crassostrea rhizophorae were collected at the mangrove of "Barra de Guaratiba" district, Rio de Janeiro, Brazil, with the aim to determine the proximate composition and fatty acid content. Along the year seasons no statistical (P>0.05) difference was observed in the values of moisture, crude protein, crude lipid and ash. They were 82.0%; 9.7%; 1.7%; 3.2%, in average, respectively. However, glycogen was significantly (P<0.05) higher in spring (4.4%) and winter (4.2%) samples, than in summer (2.7%) and autumn (2.9%), samples. Saturated fatty acids and polyunsaturated fatty acids were respectively, the most important fatty acids in oysters, with the palmitic acid (16:0), being the major fatty acid. This study, demonstrated that this species was characterized by low fat content (≤ 2.0%) and also being a good source of eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) fatty acids. Therefore, C. rhizophorae, in terms of lipid and fatty acids, could be recommended for human consumption.

Key words: Mangrove oysters, *Crassostrea rhizophorae*, proximate composition, fatty acids, year seasons, human health

INTRODUCTION

The excellent standard quality of seafood in human nutrition lies not on their high quality protein for which there are many other alternatives, but in the high content of n-3 highly unsaturated fatty acids (n-3 HUFA), mainly the eicosapentaenoic acid (20:5n-3, EPA) and the docosahexaenoic acid (22:6n-3, DHA), which are associated for the prevention of many humans diseases (Sargent and Tacon, 1999). The comprehension that the n-3 HUFA (20:5n-3; 22:6n-3) are essential for human bodily functions came much later than the comprehension that the arachidonic acid (20:4n-6, AA) was essential, by which time diets in western societies contained high levels of 18:2n-6 derived from vegetable oils, low levels of 18:3n-3 derived from green leafy vegetables, and also low levels of n-3 HUFA derived more-or-less exclusively from fish (Sargent, 1997).

Today, there are enough amount of evidence about the importance of n-3 highly unsaturated fatty acids (n-3 HUFA; C ≥ 20), the eicosapentaenoic acid-EPA, as well as of the docosahexaenoic acid-DHA, for the prevention of

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several human diseases. An imbalance between n-6/n-3 ratio in favor of n-6 could contribute to increase the risk of coronary heart disease (Simopoulos, 1991). The lack of these fatty acids have been associated with the occurrence of other diseases, including hypertension, inflammatory and immune disorders, depression and neurological disorders. There are also some evidence that DHA carry out certain function in the brain and in the retina, that cannot be done by the n-6 fatty acid series (Neuringer et al., 1988).

It is well known that the vertebrates requires the EPA, DHA and AA for normal growth and development. These fatty acids act as precursors of hormones called eicosanoids. The main precursor of eicosanoids is AA and those originated from EPA being less active. The eicosanoids are responsible and involved in many physiological functions and they are produced in response to stressful situations. High intakes of n-6 fatty acids, which are highly presented in modern Western food, lead to an increase of the eicosanoid production. However, it is known that the eicosanoids produced from EPA competes with the production of eicosanoids from AA. The appropriate balance of n-6 and n-3 fatty acids, is one the most important nutritional aspect for human health, because of the capacity of n-3 fatty acid to inhibited the production of eicosanoids originated from n-6 fatty acids (Sargent et al., 1999).

While an appropriate EPA/AA ratio is very important to prevent high production of eicosanoids, the DHA plays important functions of neural and visual tissues of humans (Sargent et al., 1999). There are some concrete evidences that human beings, fed on deficient food of this fatty acid during early life development, can result in serious visual and cognitive sub-abnormalities (Sargent,1997).

The importance of DHA in the neural development of the foetus and in post-natally has also focused the important role of this fatty acid series, in infants as well as for adult nutrition (Carlson et al., 1993; Salem and Pawlosky, 1994; Uauy et al., 1994). Actually, many formula feeds for premature and, on occasions, full-term infants are supplemented with the DHA fatty acid (International Society for the Study of Fatty Acids and Lipids, 1994). Furthermore, evidence is also emerging for a role of EPA and DHA in a considerable number of mental disorders, such as schizophrenia (Pett, 1997) and in some kinds of aggressive behavior (Okuyama et al., 1997). Add to that, Crawford et al. (1976a,b) reported the DHA was a limiting factor in the evolution of the brain. See Broadhurst et al. (2002) for a complete review of these evidences.

In modern society, there is a great concern about obesity problem. The obesity is directly related with the ingestion of certain types of fattiness food, rich in saturated fatty acids, such as the red meat. Consequently, there was a great stimulus for the production of low fat products and fat substitutes in the past years. Recent research suggests that there is a need to consider the quality and the quantity of fat in diets of western populations. Nowadays, there are a consensus that consumption of adequate levels n-3 HUFA acids are insufficient in most western diets, because the low consumption of sea food and its products, which are the main source of EPA and DHA (Willians, 2000).

Therefore, the aim of the present study was to investigate the proximate composition and the fatty acids content of the Brazilian indigenous mangrove oyster, *Crassostrea rhizophorae* along the year seasons, in order to evaluate its nutritional quality for human consumption.

MATERIALS AND METHODS

Sample collection

A total of 50-60 mangrove oysters *Crassostrea rhizophorae* were collected once a month, from November 2001 to October 2002 at the mangrove of "Barra de Guaratiba" district, Rio de Janeiro city, Brazil. Water temperature was checked at each collection using a hand mercury thermometer and ranged between 24-27^{o}C. Water salinity was also checked by the use of a hand refractometer (Aquafauna, Bio-Marine, USA) and ranged between 34-35 ‰. Immediately after collection, the oysters were transferred to the laboratory.

Sample preparation

At the laboratory the shells were cleaned and then the meat was removed of the shells. The meat was homogenized in a blender (Walita Gama, Brazil), placed in plastic bags with nitrogen and kept in freezer at –20^{o}C for subsequent analysis of proximate composition and fatty acids. Three replicates of each sample collection were used for analysis, totaling nine replicates for each year seasons.

Analytical procedures

The oysters were submitted to chemical analysis, according to standard method of AOAC (2000). Moisture was determined by drying samples in an oven (Labostar-LG 122, Japan) at 110 ^{o}C until constant weight; ash was determined in a muffle furnace (Isuzu, Japan) at 600 ^{o}C for 3h; crude protein by the Kjeldhal method (N x 6.25) using an automatic Kjeldhal system (Buchi 430/423, Switzerland). Glycogen was determined according to Plummer (1987). Oysters crude lipids were gravimetrically determined after extraction with chloroform/metanol (2:1, v/v) according to the method of Folch et al. (1957).

Oysters crude lipids were submitted to saponification with potassium hydroxide (KOH-50%) and the fatty-acid methyl esters (FAME) were prepared by esterification with boron-trifluoride in methanol (7%) (Metcalfe and Schmitz, 1961). The FAME were separated by gas-liquid chromatography on a Shimadzu GC-15A (Japan), equipped with a flame ionization detector (FID) and fitted with a fused silica capillary column (Omegawax 320 x 30 m x 0.32 mm i.d., Supelco, Belefonte, USA). Hydrogen was used as the carrier gas with a flow rate of 40 ml/min. Injector and detector temperatures were programmed to be 240 and 250^{o}C, respectively. Column temperature was programmed to be isothermal (205^{o}C). The FAME were identified by reference of known standards (Supelco) and quantified by a Shimadzu C-R4 (Japan) integrator.

Statistical analysis

Data were statistically analyzed by ANOVA to test the range of the proximate composition and fatty acid contents along the year seasons. Where significant ($P<0.05$) differences were found, a Tukey test was used to rank the groups. All statistical analyses were performed using the SAS, Version 6.8 software (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Proximate composition

No statistical difference ($P>0.05$) was observed for moisture, crude protein, crude lipid and ash (wet basis) along the year seasons. Moisture was 81.9% on average, crude protein ranged between 9.3%-10.2%, crude lipid and ash were 1.6% and 3.1 % on average respectively. However, glycogen was significantly different among the spring (4.4%) and winter (4.2%) samples, in comparison to the summer (2.7%) and autumn (2.9%) samples. Oysters proximate composition was in accordance with the proximate composition data of aquatic animals (IBGE, 1985; NRC, 1993). Results are shown in Table 1.

Table 1 - Proximate composition of the mangrove oyster *C. rhizophorae* along the year seasons.

	Year seasons			
Proximate composition[1; 2]	**Spring**	**Summer**	**Autumn**	**Winter**
Moisture	81.1±0.1	82.1±0.1	83.0±0.2	81.4±0.1
Crude protein	10.2±0.1	9.9±0.1	9.3±0.1	9.6±0.1
Crude lipid	1.5±0.0	1.6±0.0	1.5±0.0	2.0±0.1
Ash	2.8±0.0	3.7±0.1	3.3±0.1	2.8±0.1
Glycogen	4.4±0.0^{a}	2.7±0.0^{b}	2.9±0.1^{b}	4.2±0.1^{a}

[1] *Wet basis (%)*

[2] Values are means of nine replicates (n=9) ± standard error (SE). Values within a rows with the same letters are not significantly different (P>0,05).

Fatty acids

Significant differences ($P<0.05$) were observed among the fatty acids compositions of the oysters (Table 2). Saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA: n-6 + n-3) respectively, were the most important groups of fatty acids among the oysters. Palmitic acid (16:0) was the major fatty acid among the oysters samples. The sum of SFA was significantly lower ($P<0.05$) in winter than in the other seasons.

Monounsaturated fatty acids (MUFA) were the lowest fatty acid group found in the oysters. They were significantly ($P<0.05$) lower in spring, than in the other seasons. The oleic acid (18:1n-9) and the palmitoleic acid (16:1n-7) were respectively, the major MUFA found in the oysters.

Table 2 - Fatty acid content (expressed as % of total fatty acids) of the mangrove oyster *C. rhizophorae* along the year seasons.

	Year seasons			
Fatty acid[1]	**Spring**	**Summer**	**Autumn**	**Winter**
14:0	9.8±.0.2[a]	7.0±0.2[a,b]	4.1±0.1[b]	3.7±0.1[b]
15:0	1.1±0.0	1.1±0.1	0.9±0.0	1.1±0.1
16:0	15.1±0.1[a]	12.6±0.3[b]	16.0±0.2[a]	10.2±0.2[b,c]
17:0	1.3±0.1	2.2±0.1[a]	1.4±0.1	0.9±0.1
18:0	1.6±0.1[b]	2.5±0.1[a]	3.7±0.2[a]	1.2±0.1[b]
∑ SFA[2]	28.9±0.3[a]	25.4±0.3[a,b]	26.1±0.3[a,b]	17.10.4 [b]
16:1n-7	5.1±0.1[a]	2.2±0.1[b,c]	3.6±0.1[b]	1.3±0.1[c]
16:1n-9	0.2±0.0	1.1±0.1	0.7±0.1	0.4±0.1
18:1n-9	2.3±0.1[c]	12.6±0.4[a]	4.9±0.2[b]	1.3±0.1[c,d]
18:1n-7	1.0±0.1	0.5±0.1	0.9±0.1	0.6±0.1
20:1n-11	nd	2.2±0.1[a]	1.6±0.1	2.3±0.1
20:1n-9 +7	3.1 ±0.1[b]	4.6±0.2[a]	2.1±0.1[b,c]	1.1±0.1[c]
22:1	0.4 ±0.1[b]	0.5±0.1[b]	1.7±0.1[a]	1.6±0.1[a]
∑ MUFA[3]	12.1±0.1 [b]	23.7±0.4[a]	15.5±0.2 [b]	8.6±0.2 [c]
18:2n-6	1.3±0.1	1.6±0.1	1.4±0.1	0.7±0.1
18:3n-6	nd[9]	0.5±0.1	0.7±0.1	0.4±0.1
18:4n-6	0.5±0.1	0.6±0.1	0.6±0.1	0.3±0.1
18:3n-3	0.3±0.1	1.0±0.1	1.4±0.1	0.8±0.1
18:4n-3	1.8±0.1[a,b]	1.9±0.1[a,b]	2.4±0.1[a]	2.2±0.1[a]
20:3n-6	0.9±0.1[c]	0.5±0.1[b]	0.5±0.1[b]	1.7±0.1[a]
20:4n-6	1.9 ±0.1[a,b]	1.0±0.1[b]	2.6±0.1[a]	0.8±0.1[b]
20:4n-3	0.9 ±0.1[a]	1.0±0.1[a]	0.6±0.1[b]	0.4±0.1[b]
20:5n-3	10.6 ±0.1[a]	4.5±0.1[b]	9.7±0.2[a]	10.2±0.2[a]
22:4n-9	1.6 ±0.1[b]	1.8±0.1[b]	1.7±0.1[b]	3.1±0.2[a]
22:5n-3	0.5±0.1	0.3±0.0	0.6±0.0	0.7±0.1
22:6n-3	5.6±0.2[a]	2.6±0.1[c]	6.4±0.1[a]	4.9±0.1[a]
∑ PUFA[4]	24.6±0.2[a]	15.7±0.2[b]	27.2±0.2[a]	25.5±0.2[a]
∑ n-6	4.6.±0.1[b]	4.2+0.1[b]	5.8±0.2[a]	3.9±0.2[b]
∑ n-3	19.7±0.2[a]	11.3±0.2[b]	21.1±0.2[a]	19.2±0.3[a]
∑ n-3 HUFA[5]	17.6±0.3[a]	8.4±0.2[b]	17.3±0.2[a]	16.2±0.2[a]
SFA/TUFA[6]	0.8±0.1	0.6±0.1	0.6±0.1	0.5±0.1
n-6/n-3	0.2±0.0	0.4±0.0	0.5±0.0	0.2±0.0
n-3/n-6	4.3±0.1[a]	2.7±0.1[b]	3.6±0.1[a,b]	4.9±0.1[a]
DHA/EPA[7]	0.5±0.1	0.6±0.1	0.6±0.1	0.5±0.1
CL[8] (%)	1.5±0.0	1.6±0.0	1.5±0.0	2.0±0.1

[1] Values are means of nine replicates (n=9) ± standard error (SE). Values within a rows with the same letters are not significantly different (P>0.05).[2] Saturated fatty acids. [3]Monounsaturated fatty acids. [4]Polyunsaturated fatty acids.[5] Highly unsaturated fatty acids (C ≥.20). [6] Saturated fatty acids/total unsaturated fatty acids. [7] Docosahexaenoic acid/ eicosapentaenoic acid. [8] Crude lipid. [9]Not determined

The concentration of the oleic acid was higher (P<0.05) in summer than in the other seasons. The sum of PUFA was lower (P<0.05) in autumn than in the other seasons, while the sum of the concentration of the n-3 HUFA was lower (P<0.05) in summer than in the other seasons. The 20:5n-3 (EPA) and the 22:6n-3 (DHA) respectively, were the major fatty acids found among the PUFA. The n-3/n-6 ratio was higher (P<0.05) in spring and winter than in summer and autumn. Conversely, no difference (P>0.05) was observed in the n-6/n-3 and in the DHA/EPA ratios along the year seasons. Although, the concentration of PUFA were lower (P<0.05) in summer than in the other seasons, no difference was (P>0.05) observed in the SFA/TUFA. This was because of the increase (P<0.05) of the MUFA concentration in the summer. This result leads to the conclusion, that a mechanism may

exist to keep the SFA/TUFA ratio in an appropriate balance inside the animal organism.

DISCUSSION

In animal tissues carbohydrates are mainly present in the form of glycogen and they are mainly concentrated in liver. Oysters are among the animal species, which contains the highest concentration of glycogen. Glycogen values found in the oysters from the present study were similar of the values reported by IBGE (1985).

In general, most type of marine organisms are characterized by lipid levels lower than 3%. The results from the present study demonstrated that this species was also characterized by a low lipid content (≤ 2%).

Fatty acid composition of aquatic animals is influenced by intrinsic variables (sex, age, size and way of life) as well as extrinsic factors (diet, salinity and temperature). Temperature is one of these variables that much influence fatty acid composition. As temperature decreases, the level of unsaturation tend to increases to assists in maintaining the freezing point below that of surrounding water to ensure membrane fluidity and general body flexibility (Eastman, 1990; Martino et al., 2002). However, at higher temperatures, an increase of phospholipid is necessary to counteract excessive fluidity (Martino et al., 2002). This situation may partially explain the raised concentration of SFA found in the oysters musculature.

However, the SFA found in the highest concentration was palmitic acid (16:0), which has been commonly found in marine species. Ackman and Eaton (1966) considered this fatty acid as the key for many metabolic process in fish and in many other aquatic animals species.

Despite of the high water temperature observed along of this study, the EPA and DHA were found at high concentrations. The concentration of these fatty acids was similar to concentrations reported by Noffs (2002) for 15 indigenous Brazilian marine fish species. This author concluded that all the species analyzed were excellent sources of EPA and DHA. Moreover, the n-3/n-6 found in this study was similar to the ratio reported by Steffens (1997) for some species of fish from temperate regions. These species normally have a higher n-3/n-6 ratio than tropical species. As mentioned previously, an appropriate n-3/n-6 ratio is very important to keep the eicosanoids production in a perfect balance level (Sargent, 1997; Martino, 2002; Martino, 2003). The imbalance of this ratio can lead to a large number of diseases in humans.

The high concentration level of n-3 HUFA, mainly the EPA found in the oysters, and the low n-6/n-3 and SFA/TUFA ratios, must be considered an excellent nutritional characteristic of this species, because this could promote a desirable balance of the eicosanoids production. According to Simopoulos (1991), there was much evidence that our early ancestors consumed diets with n-6/n-3 ratios 1:1, and this ratio changed considerable along the centuries and now became overmuch towards n-6 polyunsaturated fatty acids. Therefore, based on the finds of the present study is possible to concluding that the nutritional composition of *C. rhizophorae*, in terms of lipid and fatty acid content, could be recommended for human consumption.

RESUMO

Com o objetivo de determinar a composição centesimal e de ácidos graxos da ostra de mangue *Crassostrea rhizophorae*, amostras foram coletadas durante um ano no manguezal localizado na Barra de Guaratiba, na cidade do Rio de Janeiro, Brasil. Nenhuma diferença estatística (P>0,05) foi observada para os valores de umidade, proteína bruta, lipídio bruto e cinza, que foram em média: 82%; 9,7%; 1,7% e 3,2%, respectivamente. Por outro lado, os valores encontrados para o glicogênio foram significativamente diferentes (P<0,05) para as amostras de primavera (4,4%) e inverno (4,2%) do que para as amostras de verão (2,7%) e outono (2,9%). Os ácidos graxos saturados e poliinsaturados foram respectivamente, os principais grupos de ácidos graxos das ostras, sendo que o ácido palmítico (16:0) foi o ácido graxo mais abundante em todos as amostras de ostras coletadas. O presente estudo demonstrou que esta espécie é caracterizada tanto por uma baixa concentração de lipídios (≤ 2,0%) como também, por uma elevada concentração dos ácidos eicosapentaenóico (20:5n-3, EPA) e docosahexaenóico (22:6n-3, DHA). Portanto, baseado no presente resultado é possível concluir que em termos de lipídios e de ácidos graxos a composição nutricional da *C.rhizophorae* é recomendável para o consumo humano.

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