Influence of seasonality on the quality of oysters from the Sado and Mira rivers

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ABSTRACT
There is a lack of knowledge about the influence of seasonality on the microbial and physicochemical quality of oysters in Sado and Mira rivers. Water, sediment, and oysters (Crassostrea angulata and Crassostrea gigas) were collected for microbiological, nutritional, and sensory analyses. The microbiological water quality and the oyster shell contamination were better during the warmer months. No seasonal effect was observed on sediments and on oyster meat. A good physicochemical and nutritional quality was also observed, with high content of poly- unsaturated fatty acids, including omega-3 fatty acids, resulting in good lipid quality indices. From the sensory evaluation, both oysters' species were well scored and presented the highest scores (4) in parameters such as cream-ivory colour, sea smell, firmness and juiciness. These attributes denote the freshness degree at the time of the tasting, reflecting the quality of the bivalve.

1. Introduction

The oyster's quality is strongly related with environment conditions and the location where the oyster's growth. Oyster can reproduce and growth attached to rocks or sediments in the natural banks. They are bivalve molluscs adapt to filter the water containing phytoplankton, suspended solids, dissolved solids, and other substances to obtain their food source. Temperature, pH, dissolved oxygen, and salinity affect the reproduction cycle and oysters' growth. Oysters are filter feeders able to ingest particles in suspension that may contain pathogenic microorganisms (Pereira et al., 2006). The oyster shell is not regular and present a high roughness when the oyster growth in the wild environment. Due to the importance of the natural oyster banks, limited catches are allowed and due to the shape of shell, they are not so attractive commercially. To answer to the market demand and to increase the quality of the oyster, they are produced in aquaculture (Forrest et al., 2009). In the aquaculture, the production technology in extensive mode takes advantage of the natural food present in the water. The oysters grow on suspended systems avoiding the contact with the sediment to improve the quality oyster. In this production process, the species are feed directly from the natural environment and the production takes advantage of the aeration, feeding and natural water renewal, allowing to the producers to have a reduced interference in the production and no need to provide additional food to the oyster. The oysters can grow on intertidal areas and in earth ponds, suspended from the sediment in bags, using long-line systems and/or over tables. This suspension methods allow to the production to avoid the direct contact with the sediment and the pseudo-faeces, which can potentially cause some dis- eases in the oysters (Forrest et al., 2009). Many shapes of the bags (e.g., rectangular or cylindrical) can be used to support the oyster growth in the aquaculture system. The advantage of using bags allows the producer to handle the bags with regular movements to improve the quality of the oyster shell, by releasing the accumulation of suspended solids and other living organisms. Additionally, it also allows to the oysters to grow individually reducing the effect of growing attached to each other. During the growing process is normal to find mortalities resulting from the climatic changes (e.g., temperature, dissolved oxygen, and salinity during the sunny and raining periods). Mortalities ranging from 30% to 50% can occur, depending on the location where the oysters are produced (Parker & Bricker, 2020). Temperature, chlorophyll concentrations, diatom biomass and potentially dangerous algae can release toxins and lead to high mortalities (Cassis et al., 2011; Forrest et al., 2009) or by diseases

caused by microorganisms (Pernet et al., 2012).

The oysters' production in Portugal is based on three main species: Crassostrea angulata (Lamarck, 1819) also called Portuguese oyster, the Ostrea edulis (Linnaeus, 1758), known as flat or European oyster, and the Crassostrea gigas (Thunberg, 1793) known as Pacific oyster. The C. angulata is an indigenous species which can survive in low and high salinities, being found in the Portuguese rivers and estuaries (Azeredo et al., 2018; Portela, 2016), and due to its high growth rates and resistance to environmental conditions changes, it is chosen for cultivation in many regions of the world (Miossec et al., 2009).

In both natural environment and aquaculture, the oysters can be contaminated by pathogens. Among the main bacteria and virus, we find Escherichia coli, Salmonella, Vibrio, Norovirus and Hepatitis A virus (Jeamsripong & Atwill, 2019; Pereira et al., 2006). E. coli can be responsible for serious food poisonings. To avoid human health problems, the oysters are depurated and purified before commercialization, by placing the oysters in clean water where they can eliminate possible chemical and microbiological contamination. The E. coli is a good indicator of a faecal contamination, and it allows evaluating the risk of contamination by other faecal pathogens.

The seasonal environmental conditions variations can affect the microbial and physicochemical quality of oysters. The study was carried out on two aquacultures in the Sado and in the oyster natural banks in Mira River, located in the southwest of Portugal. Water and sediment samples were collected to physicochemical and microbiological analyses in both locations. Oyster samples were also collected to microbiological analysis before depuration. Microbiological, nutritional, and sensory analyses were carried out on depurated and purified oysters. According to our knowledge there is a lack in literature about the relation between seasonal of the oyster environmental growth conditions and its relationship with the oyster quality, in terms of nutritional, sensory and microbiological data.

2. Materials and methods

2.1.Study area

Two aquacultures in the Sado were selected to study the water quality by physicochemical and microbiological analyses to characterize the environment conditions for the oyster aquaculture production. In the aquacultures on the Sado, the oysters had grown on bags over tables and in cylindrical floating bag system (with regular movement of the bags according to the tide height). Oysters filter the water to catch the nutrients and the food, and when it grows on sediments, and it can accumulate undesirable substances and subsequently higher content of microorganisms. For this reason, in the aquacultures, the oysters had grown on a support to avoid contact with sediments. The water in the earth ponds was continuously renewed according to the tide height, however when the temperature and salinity conditions of the water did not favor the oyster growth, the water gates of the earth ponds can be
opened or closed to avoid negative effects on the production.

A spot in Mira river (37°40'56.22"N; 8°43'49.92"O) was selected due to the high density of oysters in the natural bank and was considered to have the best environment place in the river to the oysters growth. In the Mira river, the oysters grow over the sediment in the wild environment without any human action to improve the oysters quality.

2.2.Oyster sampling

The sampling took place during low tide (±1.1 m relative to mean sea level) in both rivers. C. angulata and C. gigas triploid were obtained from two aquaculture in the Sado river and oyster samples of C. angulata were collected in natural banks in the Mira river. Random oyster samples were collected during the different season periods of the water quality monitoring. The study was carried out in Sado and Mira rivers from May 2018 to March 2019. These sampling months were selected according to suggestion and indication by the nurseries, as being the moments of highest production, being in the best quality conditions, highest consumer demand, among other reasons.

2.3. Physicochemical analyses of water samples

Monthly, water samples were collected to monitoring the local oyster growing areas in Sado and in Mira rivers during all studied periods. Water samples (1.5 L) were collected in each point. After collection, the water samples were stored in isotherm boxes at +4 °C before analysis at the laboratory.

The water temperature, salinity, dissolved oxygen, and pH were measured during the sampling collection with a multiparametric probe. The total nitrogen and phosphorus, biochemical oxygen demand (BOD) and chlorophyll-a were measured in the laboratory, according to standard methods (Baird & Eaton, 2022).

2.4. Microbiological analyses of water, sediment, and oyster samples

2.4.1. Sampling for microbiological analyses

Water, sediment, and oyster samples were collected sequentially on May, July, October 2018 and March 2019, to microbiological analyses in Sado and Mira rivers in the same locations of the physicochemical analyses.

Oyster shell and meat, with the fluid intravalvulary before and after depuration and purification processes, were analysed. The oyster, sediment and water samples were submitted to determination of total and faecal coliforms and E. coli. All the samples were collected with material previously sterilized. After collection, all the samples were stored in isotherm boxes at +4 °C until their arrival to the laboratory for analysis. The Most Probable Number (MPN) technique was used as reference method to detect and measure the total, and faecal coliforms and E. coli in the samples.

2.4.2. Water samples preparation for microbiology analyses

To analyze the water samples, five dilutions (up to 10^{-5}) were pre- pared by adding 1 mL of each water sample to inoculate each tube of a series of three tubes containing 9 mL lauryl sulphate tryptone broth. The dilutions were used to measure the total coliforms.

2.4.3. Oyster and sediment samples preparation for microbiological analyses

The oysters (n=6) were analysed before and after depuration. The oysters were washed with a brush and water to remove all material adhered to the shells in the oysters. The oysters were placed on a stainless-steel tray for air drying. The oysters were removed from their shell with a sterile stainless-steel instrument (pre-autoclaved) appropriate for oyster opening. The fluid intravalvulary and the meat inside the oyster shell were transferred aseptically to sterile bags appropriate for disintegration in a bag mixer blender. The oyster shell was also analysed separately from the meat for microbiological analysis. To the determination of Coliforms, an enrichment of the oyster (meat and shell) and sediment samples was carried out, by weighing 15 g of the sample and added to 200 mL 0.1% (wt/vol) peptone water and stirred for 24 h.

A series of ten-fold dilutions up to 10^{-5} were then prepared and 1 mL of each dilution was inoculated into each tube of a series of three tubes containing 10 mL lauryl sulfate tryptose broth (LST). The analyses were carried out in triplicate.

The dry weight of the sediment and the oysters were measured to express the data by dry weight. Five grams of wet sediment and oyster sample were weighted and then placed in the oven at 100 ° C for 48 h to remove any traces of water in the sediments and oysters, until constant weight. The determination of the dry sediment allowed establishing the relationship between the dry sediment or oyster/wet sediment or oyster.

2.5. Total and faecal coliforms and E. coli determination

2.5.1. Total coliforms

For each dilution, in five tubes, containing10 mL of brilliant green bile 2% broth (BGB) and a Durham tube, 1 mL of the dilution was transferred and incubated during 48 h at 351 ° C. After the incubation, the BGB tubes with turbidity and gas production considered positive were quantified, and the MPN of coliforms per 100 mL was determined using the MPN table. The other tubes were considered negative.

The same procedure was carried out for oyster (meat and shell) and sediment by using 1 mL of the dilutions previously prepared from the extracts. After the incubation, the BGB tubes with turbidity and gas production considered positive were quantified, and the MPN of coliforms per 100 g was determined using the MPN table. The other tubes were considered negative.

2.5.2. Faecal coliforms

To count the number of faecal coliforms, all the positive tubes of the total coliforms were seeded in the new tubes containing 10 mL of brilliant green bile 2% broth and a Durham tube. These tubes were incubated in 44±1 ° C during 48 h. BGB tubes with turbidity and gas production were quantified, and the MPN of coliforms per gram was determined using the MPN table for three tubes. After the incubation, the tubes which were blurred and which present bubbles in the Durham tube were considered positive and the other tubes were considered negative.

2.5.3. Escherichia coli

To count the number of E. coli, all the positive tubes for faecal coliforms were seeded in the new tubes containing 10 mL of tryptone water. These tubes were incubated in 44±1 ° C during 48 h. After the incubation, some drops of Indole Kovack's reagent were added in all the tubes. If a pink ring appeared at the top of the solution, the tube was considered positive (indole positive) and if it did not form this ring, the tube was considered negative.

2.6. Physicochemical analyses and nutritional quality of oyster samples

Samples of C. angulata and C. gigas were collected in the two aqua- cultures on Sado river and samples of C. angulata were collected in wild environment on Mira. Nutritional and sensory analyses were done sequentially on May, July and October 2018, and March 2019. Proximate composition, average weight, color, fatty acid profile and nutritional fat quality indices, such as atherogenic, thrombogenic and hypo- and hypercholesteromic indices, were determined in all oysters' samples.

2.6.1. Proximate composition analysis

All oyster samples were immediately transported to the laboratory, where soft tissues were separated from the shells, pooled and homogenized using a blender. Moisture and ash content were immediately determined, while protein, lipid, and crude fiber were determined after storage at – 25 ◦ C.

For moisture content, oyster samples were dried at 105 ◦C temperature in hot air laboratory dryer (Memmert UM 400 Drying Oven, Germany) until reaching a constant weight (NP 1614, 1979). Ash con- tent was determined by using muffle furnace (Nabertherm™ Drying oven TR 240, Germany) at 550 ◦C temperature for 6 h (NP 1615, 1979). Protein content of oyster sample was determined by Kjeldahl method (N x 6.25) using Kjeldahl apparatus (FOSS Kjeltec™ 8100, Denmark) and manual titration (NP 1612, 1979). Soxhlet apparatus (FOSS ST 255 Soxtec™, Denmark) was used to determine lipids content (NP 1613, 1979). Crude fiber was determined by using fiber extraction apparatus (FOSS FT 122 Fibertec™, Denmark) (first acid boiled and then alkali boiled, both at 100 ◦ C, and then washed the filters with acetone and ether) and muffle furnace (600 ◦ C for 3 h, NP 2029, 1994). Finally, total carbohydrate was determined by calculation. All analyses were made in triplicate.

2.6.2. Oyster's average weight and color analyses Per sampling, the average weight of 25 oysters was determined as a way of controlling the weight variability between oysters. Color characteristics of oyster samples were measured, in the same 25 replicates used before, by using a CR-300 Minolta spectrophotometer (Tokyo, Japan). The instrument was calibrated before each analysis with a white standard plate. L* (lightness, black = 0, white = 100), a* (redness > 0, greenness < 0), b* (yellowness > 0, blue < 0) were recorded on each sample. These color parameters were used to obtain the color difference (AE*):

$$\Delta E^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2}$$

2.6.3. Fatty acid analyses

The fatty acid composition was determined in triplicate of samples, using a method adapted from O'Fallon, Busboom, Nelson, and Gaskins, (2007). Lyophilized samples were weighted into incubation tubes (1 g), then, 1.0 mL of C19:0 methyl ester (1 mg/mL) as internal standard, 5.3 mL of methanol, and 0.7 mL of 10 N potassium hydroxide were added to each tube and incubated in a 55 ◦ C water bath for 1.5 h. After cooling in cold water, 0.58 mL of 24 N sulphuric acid was added, and tubes were incubated again in a 55 ◦ C water bath for 1.5 h. After cooling again in cold water, 3 mL n-hexane was added, and then centrifuged for fatty acid extraction.

Methyl esters were analyzed using an HP6890A gas chromatograph (Agilent, Avondale, PA, USA), equipped with a flame-ionization detector and fused silica capillary column (SP2560 100 m x 0.25 mm internai diameter x 0.20-^m film thickness, Supelco Inc., Bellefonte, PA, USA) was used. The injector and the detector were set at 250 and 280 ◦C, respectively. Helium was used as the carrier gas at a flow rate of 1.0 mL/ min. The initial oven temperature was 50 ◦C (held for 1 min), subsequently increased to 150 ◦C (at a rate of 50 ◦C/min, and held for 20 min); another rise to 190 ◦C (at a rate of 1 ◦C/min, and held for 0 min); final increase to 220 ◦C (at a rate of 2 ◦C/min) and held for 18 min. Fatty acids were expressed as % of total fatty acids.

The polyene index (PI), as a ratio of polyunsaturated to saturated fatty acids, was calculated as follows (Ortiz et al., 2014):

$$\text{Polyene index} = \frac{\text{C20 : 5} + \text{C22 : 6}}{\text{C16 : 0}}$$

2.6.3.1. Nutritional fat quality indices. The atherogenic (AI) and thrombogenic (TI) indices, as proposed by Ulbricht and Southgate (1991), were calculated according to the following equations:

$$AI = \frac{[(4 \times C14:0) + C16:0]}{(\sum n-6 + \sum n-3 + \sum MUFAn-9)}$$

$$TI = \frac{(C14:0 + C16:0 + C18:0)}{(0.5 \times MUFA)} + \left(0.5 \times \sum n-6\right) + \left(3 \times \sum n-3\right) + \left(\frac{\sum n-3}{\sum n-6}\right)$$

Where C14:0 = myristic acid, C16:0 = palmitic acid, C18:0 = stearic acid, y n – 6 = sum of omega-6 polyunsaturated fatty acids, y n – 3 = sum of omega-3 polyunsaturated fatty acids, y MUFAn – 9 = sum of omega-9 monounsaturated fatty acids, and MUFA = monounsaturated fatty acids.

To calculate the hypo- and hypercholesteromic index (hH ratio) was used the following equation (Santos-Silva et al., 2002):

$$hH = \frac{(C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)}{(C14:0 + C16:0)}$$

where C18 : 1n – 9 – oleic acid, C18 : 2n – 6 – linoleic acid, C20 : 4n – 6 – arachidonic acid, C18 : 3n – 3 – alpha-linolenic acid, C20 : 5n – 3 – eicosapentaenoic acid, C22 : 5n – 3 – docosapentaenoic acid, C22 : 6n – 3 – docosahexaenoic acid, C14:0 – myristic acid, C16:0 – palmitic acid.

2.7.Sensory analyses of oyster samples

Sensory analyses were done using the edible part of C. angulata and C. gigas samples, which were collected from two aquacultures on Sado river and samples of C. angulata from wild environment on Mira. Sensory analyses were carried out sequentially in July and October 2018, and March 2019 . The sensory analysis was carried out with oysters in “natura” and in three sessions by a group of panelists (N – 30), to whom depurated samples were provided in specific cups with ice and the oyster's shell open and ready to consume. Each panelist had each sample in an individual booth and had to evaluate descriptors of appreciation of appearance, smell, taste, and texture, using a scale of 1 (minimum in- tensity) to 5 (maximum intensity) for each descriptor. Sensory attributes of oysters were grouped into these categories: appearance (ivory cream color, brownish-yellow color, bivalve brightness, shell filled by bivalve and exudate clarity); smell (sea air, melon fruity, fruity cucumber, and sludge); taste (sweet, salty, bitter, algae, melon, cucumber, dry fruits, and metallic/copper); texture (firmness, elasticity, and juiciness) and general evaluation (persistence, and aftertaste).

2.8.Statistical analyses

Concerning microbiological analyses of water, sediment, and oyster samples, MPN returns discontinuous data and therefore non-parametric statistical analysis was used for all the MPN data. For multiple sample comparisons a Kruskal-Wallis's test with an error probability of a = 0.10 was used to compare the results between the different areas. When tests were significant for more than two areas, pairwise comparisons with an error probability of a – 0.20 were performed. Statistical tests were performed using Jamovi statistical software version 1.6 (phpBB®, 2020).

In the case of physicochemical analyses and nutritional quality of oyster samples, statistical data analysis was performed using the analysis of variance (ANOVA) and Tukey's honestly significant difference test, at a 5% level of significance. Statistical tests were performed using the Statistica software.

3. Results and discussion

3.1.Physicochemical water quality in the oyster growing areas

The physicochemical water quality was measured to characterize the oyster growing areas during the period of the study. Data collected and analysed monthly were grouped by the different seasons for Sado and Mira rivers. The data from Sado river were collected in oyster from earth pond aquacultures using suspended bags. The average, minimum and maximum value of water quality parameter variability by season in the Sado river, is presented on Table 1.

The data from Mira were collected in oyster natural banks without any human action during the oyster growing process. The average, minimum and maximum value of water quality parameter variability by season in the Mira, is presented on Table 1.

Variability was found in water quality in oyster growth areas in Sado and Mira rivers throughout the different seasons. This variability affected the oyster growth rates and the mass of the oyster meat. Ac- cording to the data, the water quality was good to oyster growth in both Sado and Mira rivers. No limiting factors were found during the studied

Table 1
Water quality in the Sado and Mira rivers (from May 2018 to March 2019) (Mean±SD; Avg: average; Min: minimum; Max: maximum).

Sado river												
Parameter	*Spring*			*Summer*			*Autumn*			*Winter*		
	Mean±SD	*Min*	*Max*	*Mean±SD*	*Min*	*Max*	*Mean±SD*	*Min*	*Max*	*Mean±SD*	*Min*	*Max*
Temp. water (°C)	22.1±3.6	19.6	24.6	18.5±0.7	22.2	23.2	15.3±2.3	12.8	18.5	14.5±3.1	11.0	17.1
Salinity (‰)	30.1±3.5	29.4	30.8	35.6±1.4	34.6	36.6	30.9±3.4	27.1	33.6	29.4±1.0	28.4	30.2
pH	7.8±1.0	7.6	8.0	7.6±0.1	7.6	7.7	7.7±0.4	7.2	8.1	7.7±0.6	7.1	8.1
Dissolved Oxygen (mg O_2/L)	6.8±0.7	6.3	7.3	5.9±0.1	5.9	5.9	8.0±5.2	2.1	11.9	10.0±2.1	8.0	12.2
Total N (mg N/L)	0.7±0.4	0.4	1.0	1.2±0.1	1.1	1.3	1.3±0.4	0.7	1.6	1.4±0.1	1.3	1.6
Total P (mg P/L)	0.6±0.1	0.6	0.6	0.3±0.1	0.3	0.3	0.2±0.1	0.2	0.2	0.7±0.9	0.2	1.7
Chlorophyll (µg/L)	24.8±10.2	17.6	32.0	26.2±22.4	10.4	42.1	59.1±26.9	40.1	78.1	18.2±20.2	4.0	41.4
BOD (mg O_2/L)	3.3±1.4	1.4	4.2	2.5±0.9	0.9	3.4	1.7±0.8	0.8	2.1	1.1±0.7	0.7	2.3
Mira river												
Parameter	*Spring*			*Summer*			*Autumn*			*Winter*		
	Mean±SD	*Min*	*Max*	*Mean±SD*	*Min*	*Max*	*Mean±SD*	*Min*	*Max*	*Mean±SD*	*Min*	*Max*
Temp. water (°C)	21.7±0.4	21.4	22.0	25.3±1.0	24.4	26.4	17.1±4.1	13.8	21.7	13.9±2.0	11.7	15.5
Salinity (‰)	16.9±2.7	15.0	18.8	24.9±1.9	22.8	26.5	24.1±6.0	18.1	30.1	20.5±1.4	19.0	21.7
pH	8.1±0.1	8.0	8.1	7.6±0.1	7.6	7.7	7.1±0.3	6.8	7.4	7.5±0.1	7.4	7.6
Dissolved Oxygen (mg O_2/L)	8.2±0.3	8.0	8.4	5.8±1.5	4.4	7.4	5.7±1.5	4.3	7.2	8.3±2.5	6.4	11.2
Total N (mg N/L)	0.7±0.1	0.7	0.7	0.9±0.6	0.2	1.3	1.0±0.4	0.6	1.5	1.4±0.5	1.0	2.0
Total P (mg P/L)	0.3±0.1	0.3	0.4	0.4±0.2	0.1	0.4	0.3±0.2	0.1	0.5	0.1±0.1	0.1	0.2
Chlorophyll (µg/L)	6.1±4.5	2.9	9.2	9.8±7.0	5.3	17.9	29.8±30.6	5.3	64.1	6.4±6.0	2.7	13.4
BOD (mg O_2/L)	3.0±0.7	0.7	4.1	1.2±0.9	0.9	2.1	0.7±0.6	0.6	1.1	1.8±0.7	0.7	3.2

Table 2
Counts of total and faecal coliforms and E. coli in water, sediment and oysters on Sado and Mira. For each microbiological parameter and river, different letters denote significant differences ($p < 0.05$) between months; Symbol (*) indicates significant differences ($p < 0.05$) between rivers for each months.

	River	Date	Total coliforms (MPN/100 mL)	Fecal coliforms (MPN/100 mL)	E. coli (MPN/ 100 mL)
Water					
	Mira	May 2018	7.9×10^{4}A	4.9×10^{3}A	< 2B
		July 2018	1.7×10^{4}AB	7.9×10^{2}B	2.2×10^{0}A
		October 2018	1.3×10^{5}B	3.3×10^{4}B	1.1×10^{1}A
		March 2019	2.1×10^{5}B	1.8×10^{4}B	1.1×10^{1}A
	Sado	May 2018	< 2 A	< 2 A	< 2 A
		July 2018	< 2 A	< 2 A	< 2 A
		October 2018	< 2 A	< 2 A	< 2 A
		March 2019	< 2 A	< 2 A	< 2 A
Sediment					
	Mira	May 2018	6.8×10^{6}AB	4.1×10^{4}AB	< 2 A
		July 2018	5.7×10^{4}A	1.2×10^{3}A	8.0×10^{1}A
		October 2018	4.1×10^{4}A	1.0×10^{3}A	3.2×10^{1}A
		March 2019	6.3×10^{6}B	1.4×10^{5}B	3.0×10^{2}A
	Sado	May 2018	8.1×10^{2}AB	< 2A	< 2 A
		July 2018	< 2 A	< 2A	< 2 A
		October 2018	< 2B	< 2 A	< 2A
		March 2019	< 2 A	< 2A	< 2A
Oyster species	**River**	**Date**	**Total coliforms (MPN/100 g)**	**Fecal coliforms (MPN/100 g)**	**E. coli (MPN/ 100 g)**
Oyster shell before depuration					
C. angulata	Mira	May 2018	1.5×10^{5}A	2.3×10^{3}A	1.8×10^{1}A
		July 2018	1.8×10^{5}AB	6.8×10^{3}A	5.3×10^{1}A
		October 2018	3.4×10^{4}B*	2.8×10^{2}A	2.2×10^{1}A
		March 2019	1.2×10^{4}AB*	3.1×10^{2}A	1.7×10^{1}A
	Sado	May 2018	2.0×10^{3}A	< 2 A	< 2 A
		July 2018	1.8×10^{3}B	< 2 A	< 2 A
		October 2018	< 2AB	< 2 A	< 2 A
		March 2019	< 2AB*	< 2 A	< 2 A
C. gigas triploid	Sado	May 2018	5.0×10^{0}A*	< 2 A	< 2 A
		July 2018	< 2A	< 2 A	< 2 A
		October 2018	< 2A	< 2 A	< 2 A
		March 2019	< 2A	< 2 A	< 2 A

Table 2 (continued)

Oyster shell after depuration					
C. angulata	Mira and Sado	May, July and October 2018, and March 2019	< 2A*	< 2 A	< 2 A
C. gigas triploid	Sado	May, July and October 2018, and March 2019	< 2 A	< 2 A	< 2 A
Oyster meat before and after depuration					
C. angulata	Mira and Sado	May, July and October 2018, and March 2019	< 2 A	< 2 A	< 2 A
C. gigas triploid	Sado	May, July and October 2018, and March 2019	< 2 A	< 2 A	< 2 A

period. Differences were found in the ranges of salinity and chlorophyll between Sado and Mira rivers. Annually, the average salinity variability was in the range of 29.4to 35.6‰ and 16.9 to 24.9‰ for Sado and Mira, respectively. The average chlorophyll values varied from 18.2 to 59.1 gg/L and 6.1 to 29.8 gg/L for Sado and Mira, respectively.

3.2.Microbiological analysis of water, sediment, and oyster

The quality of the oyster is strongly related to the water and sediment quality on the growing locations. In the aquaculture, the continuous renewal of the water and the handling of the oyster bags during the production provided the best growing conditions to the oysters. The pseudo-faeces excreted by the oysters allowing the growth of microorganisms in the sediment. Table 2 shows the coliform and E. coli data in water and sediment on Sado and Mira rivers.

The river hydrodynamic and the existence of different tributaries in the Mira river was responsible for the variability in the water and sediment microbiological contamination. When enteric bacteria are exposed to water, they are affected by a combination of various stressors such as salinity, temperature, solar radiation, oxidative stress, pH and nutrient availability. Salinity and temperature were the most important factors to influence the decrease of E. coli levels in bivalves, with a bactericidal effect (Almeida & Soares, 2012).

The microbial contamination of the water and sediment in Mira river was significantly lower when the water temperature increased ($p < 0.05$). The microbial contamination variability in the water and sediment in Mira river was mostly related with contaminated tributaries of the river responsible for the different E. Coli values found during the study.

Due to the daily renewal of the water in the earth pond on Sado river, no microbiological contamination was detected in the water. Only in the sediment, it was possible to find some slight contamination due to the oyster pseudo-faeces and sediment accumulated under the oyster bags. The suspended bag system reduced the microbial contamination of the oysters. These best practices allowed to the production to achieve the best quality of the final product.

In the Mira oyster natural banks, the oysters had grown over the sediment, in the wild environment and no handling of the oyster was carried out during all the growing process. So, the oysters had presented microbiological contamination, which had been potentially high, and the shape of the oyster shell had not been well formed. The roughness of the shell had been responsible for the accumulation of sediment; microbial growth on the oyster shell and the oyster cleaning process was more complex. Table 2 shows the coliform and E. coli data in oyster (meat and shell) samples in Sado and Mira rivers.

The production methods with the oysters growing on supported bags, the handling and the movement of the oyster bags was crucial to remove the sediments from the oyster shell and to reduce the oyster shell roughness and consequently the microbial contamination. The shell roughness accumulated more sediments, and consequently total coliforms were detected in the shell, however without any pathogenic effect.

Among oyster species from Sado river, C. angulata shells shown higher values of total coliforms in the oyster shell than C. gigas before depuration. The hygiene-sanitary quality of the oyster C. angulata, before depuration, was significantly lower than the C. gigas collected in Sado and Mira ($p < 0.05$). This difference was possibly due to the roughness of the shell and origin of oyster samples from aquaculture system and oyster natural banks, showing the importance of the handling the oysters' bags during the aquaculture production. The movement of the oyster bags during the production can reduce the roughness of the oyster shell and consequently avoid the accumulation of the sediments in the shell.

In the meat, the values of total and faecal coliforms and E. coli were inexistent (< 2 MPN/100 g (dry weight) before and after purification. The contamination of the oysters with E. coli before and after depuration collected in the different areas was not significant ($p > 0.05$). A total coliform value of < 300 MPN/100 mL of meat with fluid intravalvary is recommended to ensure the microbiological quality of the product. Molluscs with E. coli < 230 MPN/100 g of meat can be harvested for direct human consumption and if 90% samples of the molluscs contain < $4.6x10^3$ MPN/100 g of meat in E. coli and 10% of samples do not exceed $4.6x10^4$ MPN/100 g of meat in E. coli, the oysters can go for human consumption after depuration and purification in an approved plant (Florini et al., 2020). With these results, it was possible to demonstrate the high quality of the oysters in the Sado and Mira rivers. The growth of the oyster in direct contact with the sediment had shown to have a higher microbiological content. The effect of handling the oysters in the aquaculture can promote less roughness of the oyster shell becoming easier to clean and purify.

3.3. Physicochemical analyses and nutritional quality of oyster samples

3.3.1. Proximate composition

Moisture, proteins, lipids, fiber, carbohydrates, and ash content of both species of C. angulata and C. gigas, from both rivers, are given in Table S.1 (from the Supplementary material). C. angulata from Mira

river has revealed the following Chemical composition: water (70.53 - 80.09%), protein (7.19 - 17.10%), lipids (2.03 - 2.92%), fiber (0.03 - 0.69%), carbohydrates (6.10 - 24.35%) and ash (0.81 - 2.68%). In the Sado river, similar values were obtained for this species: water (64.62 - 79.49%), protein (7.75 - 9.12%), lipids (2.00 - 3.22%), fiber (0.09 - 0.49%), carbohydrates (6.78 - 30.82%) and ash (1.20 - 2.80%). For C. gigas (Sado river) was also observed a similar data: water (65.46 - 77.17%), protein (7.08 - 12.00%), lipids (2.63 - 3.55%), fiber (0.15 - 0.97%), carbohydrates (7.80 - 25.03%) and ash (1.75- 2.69%). These results indicated that the main constituent of both oyster's species is water, followed by protein, which was already observed in other studies (Asha et al., 2014). Generally, it was observed a decrease of carbohydrate content and energy values in both oyster species from both rivers during season time, resulting in the lowest values in March 2019. For the other parameters was observed some variations but without a specific trend.

3.3.2. Oyster's weight and instrumental color analyses

The average weight of 25 individuals was determined as a way of evaluating individual differences between samples (months). The results are presented in Table 3. The weight of C. angulata oyster, from Mira river, had shown a significant decrease ($p < 0.05$) from May 2018 (10.4 ±3.9 g) to March 2019 (4.3±2.0 g), similarly to those observed on the same species from Sado river, except for the last month (March 2019), which resulted in a higher value (10.6±3.8 g, $p < 0.05$). Contrarily, C. gigas did not shown significant differences ($p > 0.05$) in the average weight during the different months.

L*, a*, b* color values of oyster tissue were measured, and data are presented in Table 3. In general, the main differences were observed for C. angulata oyster from Mira River in July 2018, verifying an increase of L* value and a decrease of b* value. C. gigas did not showed significant differences ($p > 0.05$) in the instrumental color during the different months.

3.3.3. Fatty acids analyses

Fatty acids composition of C. angulata is presented in Table 4. Polyunsaturated fatty acids (PUFA) constitute the majority of the fatty acids content, followed by saturated fatty acid (SFA) and mono- unsaturated fatty acids (MUFA). The SFA content was between 30.27

Table 3
Variations of average weight (in grams, of 25 individuals) and instrumental color (L*, a* and b*) in *C. angulata* and *C. gigas* from May 2018 to March 2019. For each parameter and river, different letters denote significant differences ($p < 0.05$) between months; Symbol (*) indicates significant differences ($p < 0.05$) between rivers for each month (in *C. angulata* species).

Oyster species	River	Date	Parameter							
			Average weight (25 individuals)		*Instrumental color*					
					*L**		*a**		*b**	
C. angulata	Mira	May 2018	10.4 ± 3.9	A	65.61 ± 3.25	B	−1.28 ± 0.69	A	11.95 ± 2.36	A
		July 2018	8.2 ± 2.9	AB	97.30 ± 6.52	A*	1.84 ± 1.83	A	1.57 ± 3.85	B*
		October 2018	6.8 ± 3.1	AB	56.69 ± 5.28	B	−1.53 ± 1.22	A	11.91 ± 2.10	A
		March 2019	4.3 ± 2.0	B*	56.60 ± 6.63	B	0.93 ± 0.90	A	10.87 ± 2.71	A
	Sado	May 2018	11.7 ± 2.4	A	68.40 ± 2.33	A	−1.91 ± 1.42	A	13.81 ± 1.64	A
		July 2018	12.9 ± 2.7	A	62.06 ± 6.58	AB*	−1.43 ± 1.87	A	14.11 ± 3.13	A*
		October 2018	6.3 ± 1.4	B	58.67 ± 3.96	B	−1.48 ± 0.95	A	12.74 ± 2.38	A
		March 2019	10.6 ± 3.8	AB*	60.54 ± 5.85	AB	−1.65 ± 1.03	A	9.40 ± 2.99	A
C. gigas	Sado	May 2018	9.0 ± 2.4	A	63.95 ± 10.63	A	−1.49 ± 0.90	A	12.52 ± 2.10	A
		July 2018	14.9 ± 6.9	A	85.30 ± 22.12	A	0.38 ± 2.05	A	9.30 ± 7.62	A
		October 2018	9.0 ± 2.8	A	61.03 ± 4.32	A	−2.15 ± 1.38	A	12.16 ± 2.48	A
		March 2019	9.5 ± 2.1	A	61.17 ± 5.06	A	−0.49 ± 0.92	A	9.66 ± 2.49	A

Table 4
Variations of fatty acid composition (mg/100 g of total fatty acids) in *C. angulata*and *C. gigas*from May 2018 to March 2019.Different letters denote significant differences ($p < 0.05$) between samples (for each river); Symbol (*) indicates significant differences ($p < 0.05$) between rivers for each month, only for *C. angulata* oyster.

C. angulata																
Fatty acid (g/100 g total FAs)	**Mira**								**Sado**							
	May 2018		**July 2018**		**October 2018**		**March 2019**		**May 2018**		**July 2018**		**October 2018**		**March 2019**	
C14:0	3.92 ± 0.28	A*	3.84 ± 0.03	A*	2.27 ± 0.24	B	4.05 ± 0.09	A*	2.74 ± 0.05	B*	8.24 ± 0.14	A*	2.35 ± 0.02	BC	1.98 ± 0.03	C*
C15:0	0.60 ± 0.02	B*	0.86 ± 0.01	A	0.87 ± 0.07	A*	0.65 ± 0.04	B	0.80 ± 0.04	BC*	0.92 ± 0.01	AB	1.04 ± 0.02	A*	0.70 ± 0.05	C
C16:0	19.20 ± 0.24	C*	21.47 ± 0.13	B*	23.52 ± 0.90	A*	19.01 ± 0.45	C	21.16 ± 0.07	BC*	27.81 ± 0.23	A*	21.69 ± 0.04	B*	19.76 ± 0.04	C
C17:0	1.45 ± 0.04	B	1.76 ± 0.11	A*	1.83 ± 0.06	A*	1.19 ± 0.08	C*	1.66 ± 0.01	A	1.47 ± 0.02	AB*	1.36 ± 0.01	B*	1.45 ± 0.02	B*
C18:0	5.69 ± 0.14	C*	7.30 ± 0.04	A*	6.57 ± 0.13	B*	4.82 ± 0.05	D*	5.37 ± 0.01	B*	6.55 ± 0.05	A*	3.79 ± 0.05	C*	5.42 ± 0.06	B*
C20:0	0.08 ± 0.01	A	0.12 ± 0.06	A	0.08 ± 0.01	A	0.21 ± 0.19	A	0.07 ± 0.01	A	0.09 ± 0.01	A	0.04 ± 0.01	A*	0.09 ± 0.02	A
∑ SFA	**30.95 ± 0.16**	**B**	**35.35 ± 0.06**	**A***	**35.14 ± 1.39**	**A***	**30.43 ± 0.56**	**B**	**31.79 ± 0.08**	**B**	**45.08 ± 0.27**	**A**	**30.27 ± 0.03**	**BC***	**29.53 ± 0.13**	**C**
C16:1n-7	0.22 ± 0.01	A	0.24 ± 0.07	A	0.22 ± 0.02	A	0.14 ± 0.05	A	0.16 ± 0.03	A	0.22 ± 0.02	A	0.25 ± 0.01	A	0.14 ± 0.05	A
C16:1n-9	2.39 ± 0.12	B*	2.73 ± 0.03	B*	1.75 ± 0.10	C*	3.92 ± 0.13	A*	2.88 ± 0.08	C*	4.77 ± 0.15	B*	5.90 ± 0.13	A*	1.80 ± 0.06	D*
C17:1n-9	0.22 ± 0.03	A	0.25 ± 0.09	A	0.14 ± 0.04	A*	0.19 ± 0.04	A	0.39 ± 0.02	AB	0.41 ± 0.05	AB	0.55 ± 0.01	A*	0.24 ± 0.01	B
C18:1n-9	3.59 ± 0.09	BC*	3.82 ± 0.03	B*	3.41 ± 0.08	C*	4.22 ± 0.03	A*	3.31 ± 0.05	C*	3.25 ± 0.09	C*	4.09 ± 0.03	B*	4.90 ± 0.06	A*
C18:1n-11	3.44 ± 0.05	C*	4.74 ± 0.09	B*	4.42 ± 0.09	B*	6.51 ± 0.14	A*	4.88 ± 0.10	C*	5.28 ± 0.04	B*	6.86 ± 0.03	A*	3.80 ± 0.13	D*
C20:1	5.75 ± 0.05	C*	8.49 ± 0.01	A*	6.54 ± 0.17	B*	5.94 ± 0.01	C*	6.13 ± 0.04	B*	7.29 ± 0.01	A*	6.05 ± 0.01	B*	5.12 ± 0.10	C*
∑ MUFA	**15.59 ± 0.01**	**C***	**20.26 ± 0.30**	**A***	**16.49 ± 0.46**	**B***	**20.93 ± 0.20**	**A***	**17.75 ± 0.18**	**A***	**21.22 ± 0.01**	**C***	**23.70 ± 0.13**	**A***	**15.99 ± 0.08**	**D***
C16:2n-6	0.07 ± 0.01	A	0.11 ± 0.04	A	0.08 ± 0.03	A	0.10 ± 0.02	A	0.06 ± 0.01	A	0.07 ± 0.01	A	0.04 ± 0.02	A	0.09 ± 0.01	A
C16:2n-4	0.20 ± 0.01	A	0.19 ± 0.01	A	0.18 ± 0.01	A	0.29 ± 0.08	A	0.25 ± 0.01	AB	0.31 ± 0.05	A	0.15 ± 0.03	B	0.15 ± 0.02	B
C18:2n-6	1.91 ± 0.04	A	1.64 ± 0.01	A	2.11 ± 0.09	A*	2.19 ± 0.03	A	1.70 ± 0.01	B	1.19 ± 0.01	B	3.02 ± 0.03	A*	2.56 ± 0.49	A
C20:2n-6	0.37 ± 0.02	A*	0.37 ± 0.03	A	0.31 ± 0.01	A	0.31 ± 0.02	A	0.23 ± 0.01	B*	0.32 ± 0.01	A	0.27 ± 0.03	AB	0.33 ± 0.01	A
C22:2	3.43 ± 0.01	C*	5.33 ± 0.07	A*	3.03 ± 0.16	D*	4.82 ± 0.11	B*	3.85 ± 0.06	B*	6.18 ± 0.12	A*	3.52 ± 0.01	BC*	3.18 ± 0.09	C*
C18:3n-6	0.14 ± 0.02	A	0.11 ± 0.03	A	0.17 ± 0.04	A	0.24 ± 0.14	A	0.22 ± 0.01	AB	0.05 ± 0.02	B	0.37 ± 0.02	A	0.18 ± 0.01	AB
C18:3n-3	4.36 ± 0.04	B*	3.45 ± 0.03	C*	4.88 ± 0.26	A*	3.47 ± 0.02	C*	3.56 ± 0.06	B*	1.90 ± 0.02	D*	3.15 ± 0.01	C*	4.29 ± 0.06	A*
C20:3n-6	0.23 ± 0.01	A	0.16 ± 0.01	A	0.22 ± 0.01	A*	0.17 ± 0.04	C	0.32 ± 0.01	AB	0.16 ± 0.02	C	0.37 ± 0.01	A*	0.24 ± 0.06	BC
C18:4n-3	5.92 ± 0.05	A*	3.34 ± 0.02	D*	5.39 ± 0.19	B*	3.92 ± 0.09	C*	4.41 ± 0.05	B*	1.81 ± 0.03	D*	2.91 ± 0.05	C*	5.68 ± 0.10	A*
C20:4n-6	1.46 ± 0.02	AB*	1.34 ± 0.02	B	1.59 ± 0.07	AB*	1.65 ± 0.08	A*	2.18 ± 0.05	B*	1.58 ± 0.05	C	3.88 ± 0.01	A*	1.98 ± 0.13	B*
C20:4n-3	1.14 ± 0.02	A*	0.65 ± 0.05	C*	0.83 ± 0.05	B*	0.79 ± 0.03	B*	0.72 ± 0.01	B*	0.36 ± 0.01	C*	0.45 ± 0.01	C*	0.92 ± 0.02	A*
C22:4n-6	0.18 ± 0.01	A	0.14 ± 0.03	A	0.15 ± 0.03	A*	0.18 ± 0.01	A	0.24 ± 0.01	B	0.20 ± 0.01	B	0.35 ± 0.04	A*	0.21 ± 0.05	B
C20:5n-3 (EPA)	14.71 ± 0.15	A*	11.19 ± 0.04	C*	12.43 ± 0.48	B*	12.95 ± 0.11	B*	16.32 ± 0.03	A*	7.62 ± 0.08	D*	11.48 ± 0.02	C*	14.51 ± 0.35	B*
C22:5n-6	0.53 ± 0.03	A*	0.57 ± 0.05	A	0.57 ± 0.01	A*	0.46 ± 0.01	A	0.77 ± 0.01	A*	0.49 ± 0.07	B	0.74 ± 0.01	A*	0.57 ± 0.04	B
C22:5n-3 (DPA)	1.07 ± 0.02	A	0.92 ± 0.05	AB*	0.89 ± 0.05	B	0.91 ± 0.07	AB*	1.18 ± 0.03	A	0.76 ± 0.01	B*	1.03 ± 0.01	A	1.17 ± 0.01	A*
C22:6n-3 (DHA)	11.75 ± 0.03	A*	8.24 ± 0.05	C*	10.14 ± 0.94	B	9.44 ± 0.26	BC*	9.32 ± 0.03	B*	5.39 ± 0.21	C*	9.98 ± 0.06	B	12.74 ± 0.24	A*
∑ PUFA	**47.46 ± 0.22**	**A**	**37.73 ± 0.26**	**C**	**42.95 ± 1.94**	**B**	**41.90 ± 0.59**	**A***	**45.33 ± 0.25**	**A***	**28.40 ± 0.31**	**B**	**41.71 ± 0.19**	**C**	**48.81 ± 0.30**	**A***

C. gigas								
Fatty acid (g/100 g total FAs)	Sado							
	May 2018		July 2018		October 2018		March 2019	
C14:0	2.39±0.02	b	3.02±0.19	b	3.96±0.73	a	3.11±0.26	b
C15:0	0.73±0.08	ab	0.86±0.06	a	0.74±0.05	ab	0.66±0.08	b
C16:0	19.90±0.25	c	21.80±0.37	b	23.06±0.26	a	19.20±0.28	d
C17:0	1.29±0.04	a	1.40±0.06	a	1.33±0.13	a	1.24±0.09	a
C18:0	3.96±0.23	a	4.04±0.72	a	5.11±1.33	a	4.22±0.17	a
C20:0	0.06±0.05	a	0.05±0.01	a	0.08±0.02	a	0.10±0.03	a
∑ SFA	**28.39±0.56**	**C**	**31.31±1.30**	**B**	**34.34±0.92**	**A**	**28.62±0.22**	**C**
C16:1n-7	0.25±0.02	b	0.24±0.03	ab	0.23±0.08	b	0.35±0.04	a
C16:1n-9	2.82±1.87	ab	3.80±2.12	a	3.10±0.29	a	2.25±0.02	a
C17:1n-9	0.23±0.08	c	0.31±0.17	a	0.16±0.03	a	0.16±0.03	a
C18:1n-9	4.18±0.43	a	4.27±0.56	a	3.59±0.44	a	3.91±0.20	a
C18:1n-11	3.75±1.42	a	5.05±1.87	a	4.53±0.18	a	3.84±0.11	a
C20:1	5.11±0.78	a	5.34±0.25	a	5.59±0.55	a	4.82±0.35	a
∑ MUFA	**16.32±3.73**	**A**	**19.02±3.38**	**A**	**17.20±0.09**	**A**	**14.88±0.56**	**A**
C16:2n-6	0.08±0.03	a	0.03±0.04	a	0.04±0.02	a	0.07±0.03	a
C16:2n-4	0.13±0.05	b	0.18±0.05	b	0.32±0.06	a	0.24±0.06	ab
C18:2n-6	3.14±0.30	a	2.88±0.13	a	2.49±0.74	a	2.83±0.13	a
C20:2n-6	0.55±0.16	a	0.43±0.07	ab	0.33±0.08	b	0.51±0.07	ab
C22:2	2.86±0.71	a	2.77±0.07	a	3.04±0.98	a	2.40±0.18	a
C18:3n-6	0.20±0.03	a	0.24±0.11	a	0.24±0.04	a	0.19±0.03	a
C18:3n-3	5.11±0.18	a	4.63±0.42	ab	4.18±0.03	b	4.85±0.48	ab
C20:3n-6	0.23±0.01	a	0.25±0.05	a	0.20±0.02	a	0.24±0.03	a
C18:4n-3	6.18±1.31	a	4.83±0.18	a	4.63±0.18	a	5.79±0.99	a
C20:4n-6	1.58±0.55	a	1.67±0.43	a	1.80±0.14	a	1.70±0.35	a
C20:4n-3	1.26±0.37	a	1.04±0.23	ab	0.69±0.01	b	1.22±0.08	a
C22:4n-6	0.14±0.05	a	0.12±0.04	a	0.17±0.03	a	0.19±0.04	a
C20:5n-3 (EPA)	12.58±0.28	ab	11.93±0.51	b	13.52±0.13	a	13.54±1.24	a
C22:5n-6	0.38±0.06	b	0.48±0.04	b	0.82±0.14	a	0.93±0.28	a
C22:5n-3 (DPA)	0.99±0.04	a	0.89±0.10	a	1.04±0.13	a	1.03±0.14	a
C22:6n-3 (DHA)	12.90±2.34	ab	10.67±1.34	b	9.98±0.52	b	13.85±0.80	a
∑ PUFA	**48.28±2.92**	**A**	**43.03±1.34**	**B**	**43.49±0.36**	**B**	**49.88±0.46**	**A**

±0.03 and 45.08±0.27 g/100 g total FAs, showing the higher values ($p < 0.05$) in the months July and October 2018 in Mira river, and only
July 2018 in Sado river. Palmitic acid C16:0 (~19-27 g/100 g total FAs) was the most abundant fatty acid within this fraction, followed by stearic acid C18:0 (~4-7 g/100 g total FAs). MUFAs content was be- tween 15.59±0.01 and 23.70±0.13 g/100 g of total FAs, with individuals FAs in low proportions, between ~ 0.2 and ~ 8.0 g/100 g of totals FAs. PUFA content was the highest (41.90±0.59 and 48.81±0.30 g/100 g of total FAs) fraction presented in C. angulata oyster. However, it is important to mention that July 2018, in both rivers, resulted in the lowest values of PUFAs content, with concentrations of about ~ 38 and 28 g/100 g of total FAs in Mira and Sado rivers, respectively. PUFAs n-3 EPA (C20:5n-3) and DHA (C22:6n-3) were the prominent fatty acids, showing contents of about ~ 7-16 and ~ 8-12 g/100 g of total FAs. Previous study also indicated that oyster from a different species (Ostrea edulis) was abundant in EPA (24.81% of total FAs) and DHA (14.75% of total FAs) (van Houcke et al., 2016).

Table 4 showed the fatty acids composition of C. gigas. As was observed in C. angulata, PUFAs constitute the majority of the fatty acids content, followed by saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA). The SFA content was between 28.39 ± 0.56 and 34.34±0.92 g/100 g total FAs, showing the higher values ($p < 0.05$) in July and October 2018. Similarly, palmitic acid C16:0 (~19-23 g/100 g total FAs) was the most abundant fatty acid, and MUFAs content was between 14.88±0.56 and 19.02±3.38 g/100 g of total FAs. PUFA con- tent was between 43.03 ± 1.34 and 49.88 ± 0.46 g/100 g of total, being the most abundant FAs in this fraction the PUFAS n-3 EPA (C20:5n-3) and DHA (C22:6n-3), showing contents of about ~ 12-14 and ~ 10-14 g/100 g of total FAs. In this case, only July 2018 resulted in a significant decrease ($p < 0.05$) of PUFA content (43.03±1.34 g/100 of total FAs).

In both species, the PUFAs were the most interesting fatty acid type especially the (n-3) series dominated by EPA (20:5n-3) and DHA (22:6n- 3), being more than SFAs and MUFAs, these results were also verified in other studies (Dridi et al., 2017).

3.3.3.1. Nutritional fat quality indices. The lipid quality indices of C. angulata and C. gigas are presented in the Fig. 1 and Table S.2 (from the Supplementary material), respectively.

For C. angulata from Sado river, AI, TI and hH indices varied within the season, showing an AI value and a TI values 3-times higher in July 2018, compared to the other months. In this sampling was also observed the lowest value for hH index. This result can be explained due to the fatty acid composition, mainly in n-3 PUFA content EPA and DHA, which was already observed that decrease on this month (July 2018). By calculating the polyene index (Fig. 1), which quantify the ratio of PUFAs (C20:5 and C22:6) relative to a saturated fatty acid (C16:0), indicated the same decrease in July 2018.

For C. gigas (Table S.2 from the Supplementary material), the variation of the lipid quality indices was lower than those observed for C. angulata, verifying values between ~ 0.57-0.83, ~0.19-0.27 and ~ 1.30-182 for AI, TI and hH indices, respectively. The greatest values of AI and TI and the lowest values of hH were obtained in the same month, October 2018. PI value and PUFA/SFA ratio agreed with this behavior, showing

a decrease ($p < 0.05$) at this month. This means that the decrease of lipid quality indices was strongly correlated to the decrease on PUFAs content, mainly the n-3 EPA and DHA content.

AI is related to the risk of atherosclerosis, and the high values of AI (>1.0) indicate atherosclerosis for humans (Bobe et al., 2003).IT is related to the risk of thrombosis, and high values of IT (>1.0) are also hazardous to human health (Chakraborty et al., 2016). In the present study, only C. angulata oysters from Sado river, in July 2018, showed a value higher than 1.0 for both indices (AI and TI).

Moreover, the n-3/n-6 index was always > 1.0 which had shown the occurrence of a high proportion of n-3 PUFA overn-6 PUFA in both oyster species. The ratio between n-3 and n-6 is a very useful index for comparing the nutritional value of fish lipid due to their human health

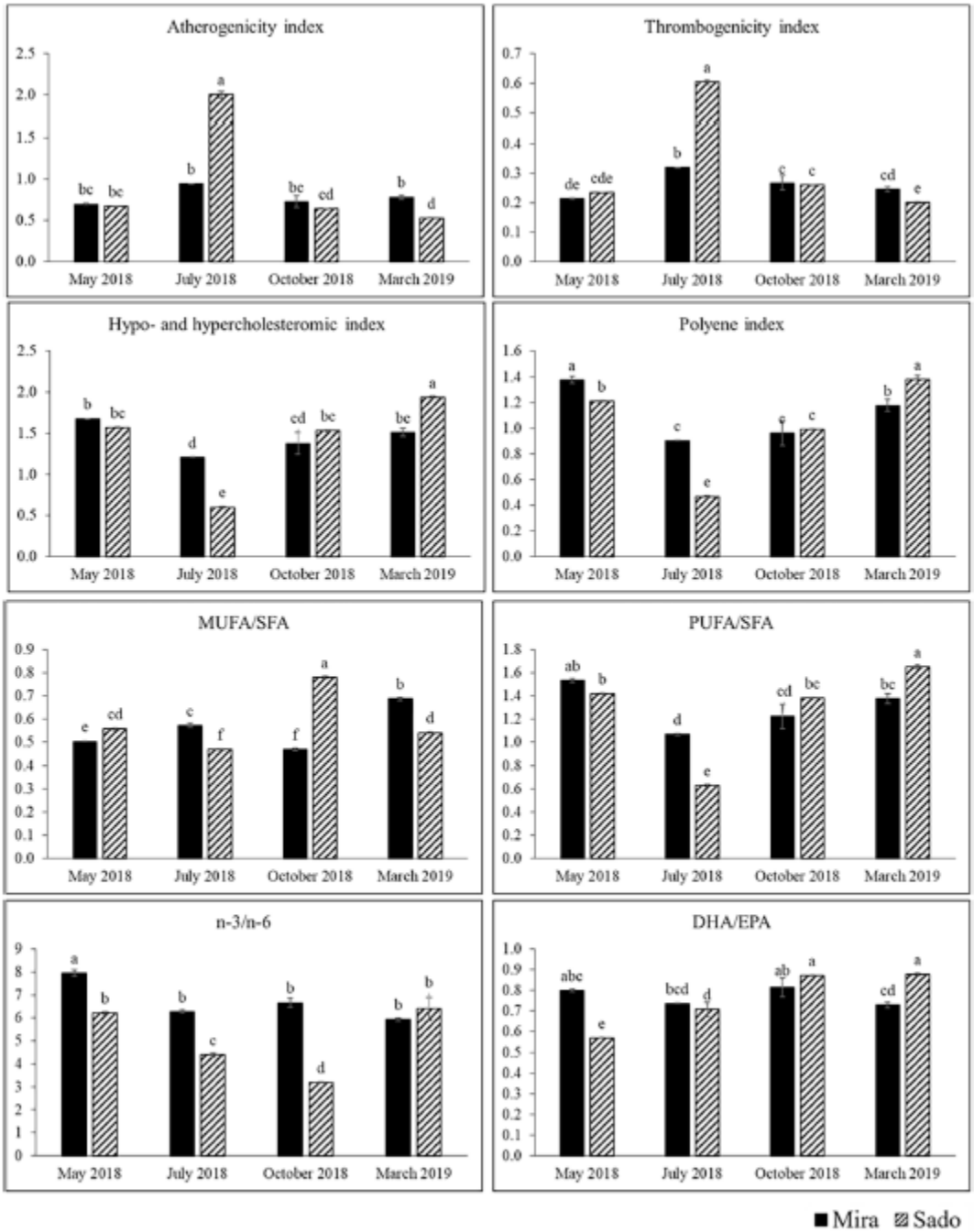

Fig. 1. Variations of atherogenicity index (AI), thrombogenicity index (TI), Hypo- and hypercholesteromic index (hH), polyene index (PI), and MUFA/SFA, PUFA/SFA, n-3/n-6 and DHA/EPA ratios in *C. angulata* in the Mira and Sado rivers from May 2018 to March 2019. Different letters denote significant differences ($p < 0.05$) between samples.

effects on coronary heart disease, cancer and autoimmune diseases (Simopoulos, 2002).

3.3.4. Sensory analyses

Fig. 2 shows the results of the sensory evaluation done by the trained panelists (N = 30) for C. angulata and C. gigas oysters. From the sensory analyses, in general terms, it was possible to verify that both oyster species were well scored, showing the highest score (4) in parameters such as cream-ivory color, sea smell, firmness, and juiciness. Specifically, in the cream-ivory color there were differences between oyster

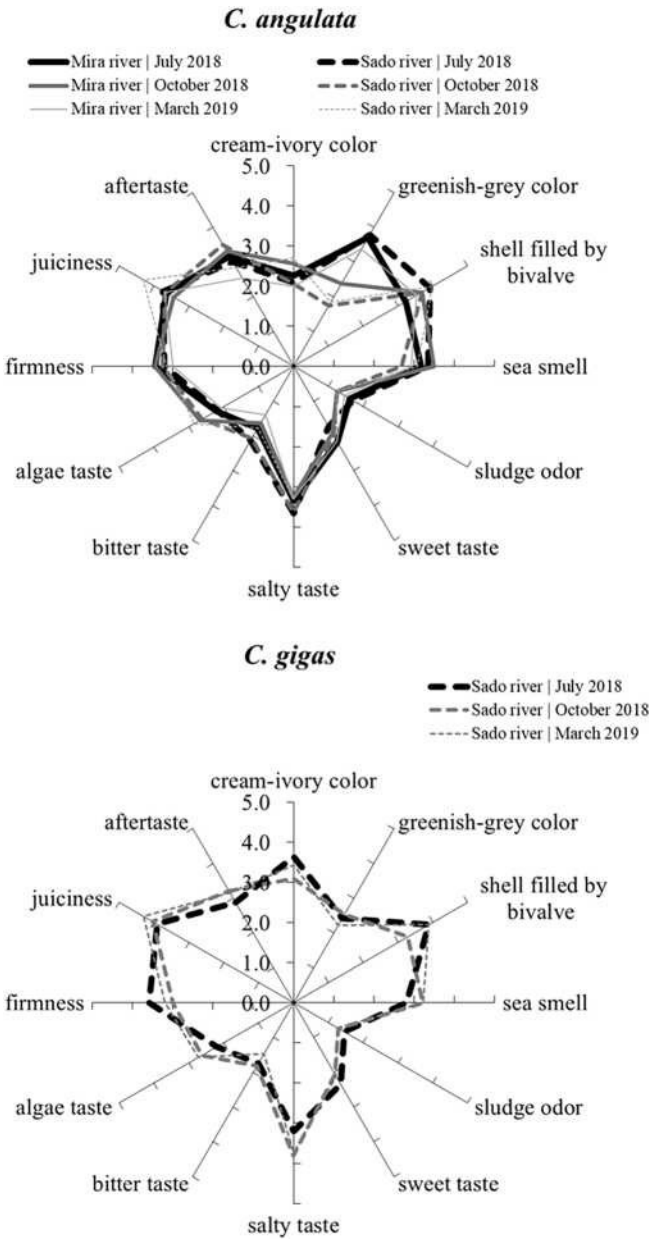

Fig. 2. Radar plot of sensory attributes of C. angulata in Mira and Sado rivers, and C. gigas in Sado river.

'species. These attributes denote the freshness degree at the time of the tasting, reflecting the quality of the bivalve. The salty taste had all very high scores (3-4) similar to those observed for sea smell and algae taste. In all samples, the odor of sludge was poorly scored (1.20-1.40), which agreed with Madigan, Kiermeier, Carragher, de Barros Lopes, and Coz- zolino (2013), with odors scored of 1.18 and 2.28 at the first and fifth days, respectively, revealing the freshness of the samples. Globally, in both species, the high scores were obtained in March 2018, and with a juiciness of 4.4 in C. gigas, followed by both July and October 2019.

In this study, both oyster species were derived from a same pro- duction area, Sado river. It has been shown by several authors (Cochet et al., 2015; Pennarun et al., 2002) that different diets and different locations of production could influence the sensory properties of oysters of the same species. So, it can therefore not be excluded that a com- parison of the sensory properties of oysters from different production areas or cultivated with different feed could lead to oysters with a more distinct flavor detectable by consumers.

4. Conclusions

The oysters found good environmental conditions for growth during the studied period, despite water quality variability observed between the different seasons. The average salinity in Mira river was lower when compared to Sado river, and the chlorophyl was higher in the Sado river when compared to Mira river in all the seasons. No limitations to the oyster growth were observed during the studied period. The microbial quality of the oyster flesh and fluid intravalvulary was good in both Sado and Mira rivers. The growth of C. angulata in Mira natural environment and in the Sado aquacultures had high roughness in the oyster shell, with consequence in a high microbial content of total coliform in the shell before depuration and purification. After depuration, no microbial contamination was observed. It was possible to show how important is the handling process of the oysters to reduce the roughness of the oyster shell and the sediment accumulation in the shell to improve the microbiological quality. The application of the best practices in the oyster aquaculture

production with the use of suspended bags can reduce the direct contact with the sediment and consequently can reduce the exposition of the oyster to microbial contamination of the sediments and improve the quality of the oysters. The microbiological water quality and the contamination of the oyster shell were better during the warmer months. No seasonal effect was observed on sediments and on oyster meat. The warmer months with higher water temperatures contribute to partially sterilise the water and reduce the water microbiological contamination. Due to changes in tidal height, oysters were exposed to the air for limited daily periods. This process, in the warmer months, allowed having an effect of reducing the microbial contamination of the oyster shell and consequently improved their quality.

Regarding physicochemical and nutritional quality of oyster samples, the results indicated that the amount in lipids and fatty acid composition varied in the different months. The analyzed oysters had a high content of unsaturated fatty acids, a good proportion of beneficial lipids for health, including omega-3 fatty acids. Among the detected fatty acids, the saturated palmitic fatty acid was the most abundant in the different months, but with lower percentages in May 2018 and March 2019. Otherwise, PUFAs had the highest levels in both oyster species.

In general, sensory analysis had shown that both C. angulata and C. gigas oysters were well scored, with the highest scores (4) in parameters such as cream-ivory color, sea smell, firmness, and juiciness. In both species, the higher scores were obtained in the March 2019 samples, and with a juiciness of 4.4 in C. gigas, followed by the July 2018 samples and the October 2018 oysters.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133292.

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