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Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins

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ABSTRACT

In this study, different physico-chemical analyses were carried out on mussels cultured at three different Spanish areas: Ría de Vigo (Galicia), the Ebro Delta (Catalonia) and Valencia Harbour (Valencian Community), in order to evaluate the influence of origin on the biometric parameters, chemical composition, and water holding capacity. Mussels from the three sites showed different proximate composition. Molluscs from Valencia showed the smallest size and the highest meat yield. All samples exhibited low concentrations of Cu and Mn, and high contents of Na, K, Ca, Mg, and P. The highest level of total free amino acids (FAA) was found in mussels from the Ebro Delta and the lowest in those from Valencia. Taurine was the main FAA in all mussels. Saturated fatty acids predominated over monounsaturated and polyunsaturated ones. Contents of palmitic acid were the highest in all samples. Galician mussels exhibited the highest amount of eicosapentaenoic acid and the lowest of docosahexaenoic acid. Mussels from the three sites showed a characteristic volatile fraction which conferred a typical flavour depending on their origin.

1. Introduction

Mussels are commercially valuable species, easy to cultivate or collect in coastal areas. They are very important for marine ecology and for human diet, since they are an important source of nutrients. Consumption of these bivalve molluscs provides an inexpensive source of protein with a high biological value, essential minerals and vitamins (Astorga-España, Rodríguez-Rodríguez, & Díaz-Romero, 2007; Karakoltsidis, Zotos, & Constantinides, 1995).

Mussels are the most representative mollusc on the Spanish bivalve market and an important resource in the Spanish fishing industry (Font, Vélez, del Río-Celestino, de Haro-Bailón, & Montoro, 2007). *Mytillus galloprovincialis* Lmk. is the only species of the genus in both the Atlantic and the Mediterranean coastal areas of the Iberian Peninsula (Sanjuan, Quesada, Zapata, & Álvarez, 1990). According to the FAO, the annual world production of mussels is approximately 1,500,000 t. The 40% of this figure is produced in the EU (600,000 t), and in Spain approximately the half of the EU production. In this way, Spain is the second producer of mussels in the world, after China (Franco-Leis, 2006). Galicia (NW Spain) represents 95% of the total mussel production in Spain; the remaining 5% is produced especially in Catalonia (NE Spain) and to a lesser extent in the Valencian Community (E Spain) (Font et al., 2007). 65% of the mussels produced in Galicia are processed (canned or frozen) and the rest are sold fresh. In spite of these percentages, fresh mussels contribute almost twice as much to the overall turnover. 70% of fresh mussels are sold in the Spanish market and the rest is exported, mainly to Italy and France (Franco-Leis, 2006). Mussel production in Catalonia and Valencia takes place mainly in the summer months, and it is consumed fresh for the most part in Spain.

There are a large number of studies on mussels from Galicia that analyze composition, growth, reproduction, physiology or parasitology; however, the literature on Ebro Delta and Valencian mussels is sparse (Gómez-Sintes, Fuentes, Fernández-Segovia, Serra, & Escriche, 2004; Ramón, Fernández, & Galimany, 2007).

In numerous studies the influence of the different environmental and nutritional conditions on the composition of mussels has been proven (Astorga-España et al., 2007; De Moreno, Pollero, Moreno, & Brenner, 1980; Fernández-Reiriz, Labarta, & Babarro, 1996; Khan, Parrish, & Shahidi, 2006; Regoli & Orlando, 1994; Szefer, Kim, Kim, Kim, & Lee, 2004).

A sensory study carried out on mussels from Galicia, the Ebro Delta and Valencia by panellists from the Valencian Community, showed that consumers found significant differences in the sensory attributes between mussels from the different origins (Gómez-Sintes et al., 2004). This fact is of particular relevance for quantifying the differences in mussel composition and other parameters that best define their quality, when cultured at these three sites. In Spain there is an Origin Appellation only for Galician mussel (MAPA. Ministerio de Agricultura, 2008). From the study of the characteristics of mussels from Galicia, the Ebro Delta and

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Valencia, it could be established the possibility of applying for an Origin Appellation for the other two areas.

Among the different parameters of fish quality, biometric measurements and chiefly meat yield are important aspects of mussel marketability. The free amino-acid profile, the fatty acid composition or the volatile fraction, are other important parameters that may be affected by the origin of mussels. The importance of amino acids in fish has also been well established from the point of view of nutrition, fish meal production, and fish flavours (Konosu & Yamaguchi, 1982; Fuke, 1994). The composition of polyunsaturated fatty acids in mussels is biologically important, since they are associated with reduced risk of cardiovascular disease (Krombout, Bosschieter, & Lezenne, 1985). Volatile compounds are responsible for the aroma of food products, one of the most important parameters in the evaluation of fish quality.

The aim of this study was to evaluate the influence of the origin on the biometric parameters, chemical composition, and water holding capacity of mussels from three different Spanish sites.

2. Materials and methods

2.1. Sample preparation

Mussels (*Mytilus galloprovincialis*) of commercial size, cultured at three different Spanish sites: Ría de Vigo in Galicia (NW Iberian Peninsula), the Ebro Delta in Catalonia (NE Iberian Peninsula) and Valencia Harbour (E Iberian Peninsula) were used as raw material. Samples were purchased in July 2002 in a local market (Mercavalencia), except mussels from Valencia, obtained from a hatchery in Valencia harbour. Samples from Galicia and the Ebro Delta were caught a maximum of 48 h before they were purchased, while this time was 24 h for mussels from Valencia. One batch from each origin was used in the study. Batches from Galicia and the Ebro Delta consisted of mussels randomly selected from the different pouches that arrived at Mercavalencia on the sampling day. The batch from Valencia was made up of samples randomly selected from different mussel rafts. The samples were immediately transported to the laboratory under refrigeration and stored at 4 °C. The analyses were performed in the same day.

2.2. Biometric parameters and meat yield

Length (maximum measure along the anterior-posterior axis), width (maximum lateral axis), and height (maximum dorsoventral axis) of 20 randomly selected mussels, were measured using a 0.05 mm precision calliper. After the biometric measurements were taken, the mussels were weighed, opened by cutting the adductor muscle with a scalpel, and the wet meat and shells were weighed. Meat yield, an important aspect of marketability of mussels, was calculated as follows: (wet meat weight/whole mussel weight) × 100 (Okumus & Stirling, 1998).

2.3. Proximate composition, minerals analysis and water holding capacity (WHC)

Moisture, total lipid, protein and ash contents were determined according to the AOAC (1997) methods 950.46, 991.36, 928.08, and 920.153, respectively. The analyses of Ca, Mg, Na, K, Mn, Cu, Fe, and Zn were performed, by flame atomic absorption spectrophotometry using a 3110 Perkin Elmer AAS (Norwalk, CT., USA). *P* was analyzed by UV–vis spectrophotometry (Escriche & Camacho, 1998) using a CE 1020 Series UV/vis spectrophotometer (Cecil Instruments Ltd., Cambridge, UK).

The WHC of fish muscle was determined in triplicate by centrifugation using a Medifriger BL centrifuge (JP Selecta, S.A., Barcelona, Spain), at 500*g* for 10 min at 10 °C, as described by Gómez-Guillén, Montero, Hurtado, and Borderias (2000).

2.4. Free amino acids analysis (FAA)

This analysis was undertaken by HPLC with pre-column derivatization using phenylisothiocyanate (PITC).

Extraction of the FAA was conducted according to the method described by Bugueño, Escriche, Serra, and Restrepo (1999) with some minor modifications. Ten grams of fish muscle were homogenized with 50 mL of 0.1 M hydrochloric acid for 3 min using an Ultra-Turrax T 25 model (IKA-Labortechnik, Staufen, Alemania). The homogenate was centrifuged at 2000*g* for 10 min at 4 °C and the supernatant was filtered through a wool glass. Deproteinization was done by combining a 1 mL aliquot of the supernatant with 2 mL of acetonitrile and centrifuging at 1600*g* for 10 min at 4 °C. A 350 μL aliquot of the supernatant was mixed with 20 μL of 1 mM α-aminobutyric acid solution (internal standard) and shaken for 30 s, and then 50 μL were taken for derivatization.

The 50 μL aliquot of the sample extract was combined with 40 μL of ethanol–water–triethylamine (TEA) (2:2:1, v/v/v), and vacuum dried in conical shaped tubes for 40 min at −40 °C in a freeze dryer (TELSTAR LIOALFA-6, Terrassa, Spain). Derivatization took place by addition of 40 μL of ethanol–water–TEA–phenylisothiocyanate (PITC) (7:1:1:1, v/v/v/v) to the tube samples. The tubes were sealed and left to stand for 20 min at room temperature before vacuum drying again. Once derivatized, 400 μL of 4 mM sodium phosphate, pH 7.4, containing 5% acetonitrile were added as a diluent and filtered through a 13 mm 0.45 μm nylon filter membrane (Waters, Mildford, MA, USA).

The derivatized amino acids were analyzed by reverse-phase HPLC in a Waters LC Module I Plus liquid chromatograph with two pumps (model W600), an auto-sampler (model W715) and a variable wavelength UV/vis detector (model W486) (Mildford, MA, USA), connected to a SpectraSystem SCM1000 membrane degasser (Thermo Separation Products, San Jose, CA, USA). Data were collected, stored and analyzed using the MILLENIUM software version 32 (Mildford, MA, USA). Separations were achieved on a reverse-phase Nova Pak C18 (3.9 × 150 mm, 4 μm particle size) (Waters, Mildford, MA, USA), thermostated at 40 ± 1 °C.

The solvent system consisted of two eluents: 0.14 M sodium acetate containing 0.5 mL/L TEA, adjusted to pH 6.4 with glacial acetic acid, and containing 6% of acetonitrile (mobile phase A); acetonitrile–water 60:40 (v/v) (mobile phase B). Mobile phases were always filtered through a 0.45 μm nylon 4700 membrane (Waters, Mildford, MA, USA). The gradient used is shown in Table 1. The injection volume was 20 μL. The chromatograms were monitored at a wavelength of 254 nm.

Identification was carried out by comparison of retention times of unknowns with authentic compounds (Sigma–Aldrich, St. Louis, MO, USA) run under the same conditions and by standard addition

Table 1
Elution program for HPLC analysis

Time (min)	Flow (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0	1.0	100	0
105	1.0	54	46
10.5	1.0	0	100
11.5	1.0	0	100
12.0	1.5	0	100
12.5	1.5	0	100
20.0	1.5	100	0
20.5	1.0	90	10
25.0	1.0	90	10

A, 0.14 M sodium acetate containing 0.5 mL/L TEA (adjusted to pH 6.4), and containing 6% of acetonitrile.
B, acetonitrile–water 60:40 (v/v).

or "spiking" (Johnson & Stevenson, 1978). Amino acid standard solutions with the internal standard were prepared and derivatized following the same procedure described for the samples.

Quantification was undertaken using calibration curves of peak area ratios (compound/internal standard) *vs* concentration ratios (compound/internal standard), under identical chromatographic conditions.

2.5. Fatty acids analysis

Fatty acids were determined by gas chromatography–mass spectrometry (GC–MS). The extraction of the total lipid was carried out according to the method described by Folch, Less, and Stanley (1957), using a chloroform-methanol (2:1, v/v) solvent system with 0.05% BHT. Transmethylation was carried out using methanol–hydrochloric acid–dimethoxypropane (40:4:1.6, v/v/v).

The derived fatty acid methyl esters (FAMEs) were analyzed using a gas chromatograph/mass spectrometer (GC–MS) Finnigan TRACE MS (TermoQuest, Austin, USA). Two microlitres of each extract were injected into a DB-WAX fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific INC., Folsom, CA, USA). Helium gas (ultrahigh purity grade, 99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min. Injector temperature was 220 °C; oven temperature was programmed from 85 to 180 °C at a ramp rate of 5 °C/min; with initial and final hold times of 2 and 5 min, respectively; oven temperature was then further increased to 240 °C at 10 °C/min and held for 5 min. The MS interface temperature was set at 220 °C, the ion source temperature was set at 200 °C, and the ionization voltage was 70 eV; the m/z range was 35–450 and the scan rate was 0.4 scans/s.

Fatty acids in the fish lipid samples were identified by comparison of retention times and mass spectra of unknowns with authentic compounds (Sigma–Aldrich, St. Louis, MO, USA) run under the same conditions. Fatty acid standard solutions were prepared and derivatized following the same procedure described for the samples. Peak areas of FAMEs standards were used to quantify by means of an external calibration curve.

2.6. Volatile compounds analysis

Analysis of volatiles was carried out by GC–MS. The isolation of volatile compounds was performed by combined simultaneous distillation-extraction (SDE) technique (Godefroot, Sandra, & Verzele, 1981) in a J&W Simultaneous Steam Distillation-Extraction Apparatus from Fisher Scientific UK Ltd (Loughborough, Leics., England) and according to the methodology described by Fernández-Segovia, Escriche, Gómez-Sintes, Fuentes, and Serra (2006) with some minor modifications. For each analysis, 100 g of sample homogenized in an Ultra-Turrax with 200 mL of bi-distilled water and 40 μL of camphor (internal standard) aqueous solution at 300 μg/mL, were put into a 500 mL round-bottom flask. The flask was held in an ultrasonic bath for 2 min to totally disintegrate the sample and it was then introduced into the oil bath of the extraction equipment and heated to boiling point. A 50 mL heart flask containing 3 mL of pentane was introduced into a water bath at 50 °C. The steam from both flasks was condensed in the common refrigerated "U-tube" of the equipment. After 1 h distillation, the content of the U-tube was collected in an airtight sealed tube. To separate the organic solvent with the volatile compounds, the tube was frozen at −18 °C; at this temperature the organic phase is liquid and has lower density than water. The liquid phase was transferred to an eppendorf tube and concentrated under nitrogen stream up to a final volume of approximately 100 μL.

The analysis was conducted on a gas chromatograph/mass spectrometer (the same equipment used for FAMEs analysis). Five microlitres of each extract were injected in split mode (split ratio 1:10) into a DB-5 fused silica capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness; J&W Scientific INC., Folsom, CA, USA). Helium gas (ultrahigh purity grade, 99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min. Injector temperature was 250 °C; oven temperature was programmed from 60 to 171 °C at a ramp rate of 5 °C/min; with initial and final hold times of 5 and 34 min, respectively; oven temperature was then further increased to 220 °C at 10 °C/min and held for 10 min. The MS interface temperature was set at 220 °C, the ion source temperature was set at 200 °C, and the ionization voltage was 70 eV; the m/z range was 35–450 and the scan rate was 0.4 scans/s.

Positive identifications were based on comparison of retention indices (RI) (van den Dool & Kratz, 1963) and mass spectra of unknowns with authentic compounds (Acros Organics, Geel, Belgium). Tentative identifications were based on comparison of RI and mass spectra of unknowns with those in the literature (Fan & Qian, 2005; Kondjoyan & Berdagué, 1996; Krist, Stuebiger, Unterweger, Bandion, & Buchbauer, 2005; Medina et al., 2005) and NIST mass spectral library, respectively.

Positively identified compounds were quantified using the same method described for amino acid quantification.

All analyses were done in triplicate, except biometric measurements and meat yield, which were performed on 20 individuals.

2.7. Statistical analysis

Data are reported as means ± standard deviation. Statistical treatment of the data was performed using the Statgraphics Plus software version 5.1 (Manugistics, Rockville, MD, USA). For each parameter evaluated an analysis of variance (one-way ANOVA) was conducted to test if there were significant differences between the three sites. The LSD procedure (least significant difference) was used to test for differences between means at a 5% significance level.

A stepwise discriminant analysis was also performed using Wilks' lambda as the statistical selection criterion for the variables. The analysis was undertaken considering volatile compound concentrations as variables and origin as the classification factor.

3. Results and discussion

3.1. Biometric parameters and meat yield

Table 2 shows the biometric measurements of the mussel shells from the three origins studied, as well as meat yield and shell percentage. Valencian mussels showed statistically smaller length ($p < 0.01$), lower shell percentage and higher meat yield when compared with the other origins. All samples showed high meat yield comparing with other studies on Mediterranean mussels where the maximum value reached was 25.2% (Vernocchi, Maffei, Lanciotti, Suzzi, & Gardini, 2007).

The differences found in the biometric parameters have a direct influence on the aspect of the product. Although Valencian mussels were the smallest, in a sensory study on these three samples carried out with panellists from the Valencian Community, they did not find differences in the aspect of cooked mussels between Galicia and Valencia. These samples had higher marks for this attribute than those from the Ebro Delta (Gómez-Sintes et al., 2004). This could be due to the lower meat yield of this last sample.

3.2. Proximate composition, minerals analysis and WHC

The proximate composition and mineral content of samples are shown in Table 2. In general, high moisture contents were found in all samples, mussels from Galicia showing the lowest value (79%). The lowest ash content also was found in this sample (2.2%),

Table 2
Biometric measurements, meat yield, proximate composition, mineral contents and water holding capacity (WHC) in mussels from Galicia, the Ebro Delta, and Valencia

Origin	Galicia	Ebro Delta	Valencia	α
Biometric measurements[y]				
Length (mm)	77 (9)[a]	72 (8)[a]	54 (5)[b]	**
Width (mm)	30 (5)[ab]	36 (4)[b]	30 (2)[a]	*
Height (mm)	22 (3)[a]	15 (3)[b]	22 (2)[a]	**
Meat yield (%)[y]	31 (6)[ab]	26 (7)[b]	34 (6)[a]	ns
Shell percentage (%)[y]	61 (8)[a]	56 (10)[a]	52 (10)[a]	ns
Proximate composition[z]				
Moisture (g/100 g)	79 (2)[a]	83.81 (0.09)[b]	81.5 (0.6)[b]	**
Ash (g/100 g)	2.2 (0.9)[a]	3.37 (0.07)[b]	3.38 (0.05)[b]	*
Fat (g/100 g)	1.4 (0.4)[a]	1.7 (0.2)[a]	2.10 (0.03)[b]	**
Protein (g/100 g)	10 (2)[a]	6.5 (0.6)[b]	10.0 (0.6)[a]	**
Mineral contents[z]				
Ca (mg/100 g)	40 (5)[a]	37 (6)[a]	82 (4)[b]	**
Mg (mg/100 g)	56 (9)[a]	41 (1)[a]	91 (10)[b]	**
Na (mg/100 g)	218 (90)[a]	415 (32)[b]	449 (111)[b]	*
K (mg/100 g)	36 (1)[a]	111 (7)[b]	139 (5)[c]	**
Mn (mg/100 g)	0.044 (0.005)[a]	0.17 (0.06)[b]	0.17 (0.02)[b]	**
Cu (mg/100 g)	0.15 (0.02)[a]	0.10 (0.04)[b]	0.12 (0.01)[ab]	ns
Fe (mg/100 g)	1.0 (0.1)[a]	2.1 (0.1)[b]	2.9 (0.5)[c]	**
Zn (mg/100 g)	2.3 (0.7)[a]	2.0 (0.1)[a]	1.76 (0.06)[a]	ns
P (mg/100 g)	314 (95)[a]	149 (70)[b]	239 (18)[ab]	ns
Water holding capacity[z] (g H_2O held/100 g H_2O)	99.6 (0.1)[ab]	99.68 (0.07)[a]	99.46 (0.09)[b]	*

Numbers in parentheses represent standard deviation. Means followed by the same letter within the same row are not significantly different, and different letters indicate significant differences.
ns, non significant; * $p < 0.05$; ** $p < 0.001$.
[y] Mean from 20 measurements.
[z] Mean from three analyses.

whereas non significant differences were found between mussels from the Ebro Delta and Valencia harbour, both exhibiting higher contents than those in literature (King, Childs, Dorsett, Ostrander, & Monsen, 1990; Orban, Di Lena, Nevigato, Casini, Marzetti, & Caproni, 2002). The lipid content was slightly higher in mussels from Valencia, while there were insignificant differences between the other two sites. Regarding protein, contents in samples from the Ebro Delta were significantly lower compared with the other two samples and with protein contents quantified by other authors (King et al., 1990; Orban et al., 2002).

The levels of the 9 minerals determined are shown in Table 2. In general, the concentrations of the minerals were similar to those found in other studies on mussels (Astorga-España et al., 2007; Hernández-Hernández, Medina, Ansuátegui, & Conesa, 1990; Karakoltsidis et al., 1995; King et al., 1990). Ca and Mg levels were significantly higher in Valencian mussels. Na, K, Mn, and Fe contents in samples from the Ebro Delta and Valencia were very similar and significantly higher than contents in mussels from Galicia. There were insignificant differences in the Cu, Zn, and P contents between the three sites. The slight differences found in the mineral content may be due to the different origin and size of mussels, as has been stated by several authors (Astorga-España et al., 2007; Regoli & Orlando, 1994; Szefer et al., 2004). These results agree with other studies that reported mussels to be a good source of calcium, zinc, magnesium, phosphorous and iron (Karakoltsidis et al., 1995; King et al., 1990).

Regarding the WHC, samples from the three origins exhibited values of this parameter above 99% (Table 2). These WHC values imply a high juiciness of the meat mussel.

3.3. Free amino acids analysis (FAA)

Nineteen FAA were identified and quantified in all samples. Table 3 shows concentrations of the FAA as well as the taste thresholds found in the literature (Kato, Rhue, & Nishimura, 1989), and the taste values (compound concentration divided by taste threshold) (Cha, Kim, & Jang, 1998), calculated for each compound. Total FAA in mussels from Valencia (3451.4 mg/100 g on a dry weight basis) was approximately two times lower than those in samples from the Ebro Delta (7153 mg/100 g) and this amount also was higher in Galician samples (5780 mg/100 g). These differences could be due to the proteolysis that might have occurred to a lesser extent in the samples from Valencia. This can be explained by the shorter time from harvesting at origin to their arrival at our laboratory, in the case of Valencian samples that were collected directly from the hatchery.

Taurine was the predominant FAA in all the samples, accounting for 79.55, 75.8, and 62.2% of the total FAA in the Galician, Ebro Delta, and Valencian samples, respectively. These results are in agreement with previous studies of blue mussel and other fish species (Cha et al., 1998; Hwang, Chen, Shiau, & Jeng, 2000; Saito & Kunisaki, 1998). Sarwar and Botting (1990) also reported that this is the major free intracellular amino acid in animal tissues. Although taurine has been reported to have no important effect on the formation of aroma active components, it has been recognised as playing an important role in human physiological functions (Fuke, 1994; Konosu & Yamaguchi, 1982). Taurine abundance was followed by glycine, arginine and alanine in all samples. The ANOVA showed significant differences in the FAA profile according to origin, except for glutamic acid, treonine and valine contents. These differences might be due to different environmental and nutritional conditions, as has been established in previous studies (Hwang et al., 2000).

The highest taste values calculated in the three samples corresponded to aspartic acid (sour) and glutamic acid (a known flavour enhancer), followed by arginine (bitter), glycine and alanine (sweet). Cha et al. (1998) found the same results in blue mussel. The high concentration of the FAA found in all samples would contribute to the intense odour of these seafood products.

3.4. Fatty acids analysis

The fatty acids found in mussels from the three origins are shown in Fig. 1a (saturated and monounsaturated) and Fig. 1b (polyunsaturated or PUFA). The saturated fatty acids accounted for 33.77, 28.47, and 39.28% of the total fatty acids in samples from Galicia, the Ebro Delta, and Valencia, respectively, predominated over the monounsaturated (17.9, 19.69, and 18.45%, respectively) and polyunsaturated (28.17, 25.46, and 31.53%, respectively). Karakoltsidis et al. (1995) found a similar percentage of saturated (36%) and polyunsaturated (29%) fatty acids in mussels. Although other authors (Freites, Fernández-Reiriz, & Labarta, 2002a, 2002b; King et al., 1990; Orban et al., 2002; Otles & Sengor, 2005) have reported that the PUFA in mussels predominated over the saturated ones, a wide range of variation in the percentages of these compounds in this mollusc have been detected, ranging from 29% to 48% for PUFA, 16–32% for monounsaturated and 23–45% for saturated ones, even values of 57% of saturated fatty acids in mussels have been reported (Pastoriza, Gallardo, Franco, & Sampedro, 1981). This variability is due to the fact that the lipid levels and composition of marine bivalves depend on the biochemical and environmental conditions of seed development (De Moreno et al., 1980). Thus

Table 3
Free amino acid (FAA) concentrations (mg/100 g in dry basis), taste thresholds found in the literature (Kato et al., 1989) for each amino acid and the taste values (compound concentration divided by taste threshold), in mussels from Galicia, the Ebro Delta, and Valencia

	FAA[z] (mg/100 g) db				Taste thresholds (g/dL)	Taste values		
	Galicia	Ebro Delta	Valencia	α		Galicia	Ebro Delta	Valencia
Aspartic acid	87 (4)[a]	159 (20)[b]	85 (25)[a]	**	0.003	29	53	28.33
Glutamic acid	156 (3)[a]	179 (27)[a]	171 (9)[a]	ns	0.005	31.2	35.8	34.2
Asparagine	51 (6)[a]	85 (27)[b]	42 (3)[c]	**	0.1	0.51	0.85	0.42
Serine	111 (58)[a]	124 (54)[a]	39 (6)[b]	*	0.15	0.74	0.83	0.26
Glycine	351 (36)[a]	763 (357)[b]	392 (75)[a]	*	0.13	2.7	5.87	3.02
Histidine	56 (10)[a]	94 (85)[b]	54 (12)[a]	*	0.02	2.8	4.7	2.7
Arginine	572 (53)[a]	338 (77)[b]	302 (46)[b]	*	0.05	11.44	6.76	6.04
Taurine	3634 (1128)[a]	4287 (642)[a]	1778 (156)[b]	**	N/A			
Treonine	33 (3)[a]	36 (9)[a]	37 (4)[a]	ns	0.26	0.13	0.14	0.14
Alanine	234 (28)[a]	268 (111)[a]	171 (13)[b]	*	0.06	3.9	4.47	2.85
Proline	38 (7)[a]	182 (30)[b]	70 (7)[a]	**	0.3	0.13	0.61	0.23
Tyrosine	63 (2)[a]	87 (6)[b]	44 (11)[c]	**	N/A			
Valine	27 (8)[ab]	34 (7)[b]	20.4 (0.7)[a]	ns	0.04	0.68	0.85	0.51
Methionine	32 (6)[a]	77 (6)[b]	24 (1)[a]	**	0.03	1.07	2.57	0.80
Isoleucine	93 (6)[a]	96 (16)[a]	53 (2)[b]	*	0.09	1.03	1.07	0.59
Leucine	21 (6)[a]	42 (2)[b]	20 (6)[a]	*	0.19	0.11	0.22	0.11
Phenylalanine	16 (1)[a]	36 (8)[b]	13 (1)[a]	**	N/A			
Tryptophan	48 (10)[a]	142 (50)[b]	57 (11)[a]	*	N/A			
Lysine	157 (2)[a]	124 (8)[b]	79 (12)[c]	**	0.05	3.14	2.48	1.58
Total	5780	7153	3451.4					

Numbers in parentheses represent standard deviation. Means followed by the same letter within the same row are not significantly different, and different letters indicate significant differences. ns: non significant; $^{*}p < 0.05$; $^{**}p < 0.001$; N/A: not available.
[z] Mean from three analyses.

fatty acid composition depends on the season, origin, and zone of culture, among others; these factors determine the environmental conditions, the phytoplankton resources available and therefore the composition of nutrients (Fernández-Reiriz et al., 1989).

In the case of saturated fatty acids (Fig. 1a), stearic acid (18:0) made an important contribution to this fraction, although in a lesser proportion than palmitic acid (16:0), clearly predominant in all samples. Palmitic acid has been reported in numerous studies, as the major saturated fatty acids in mussels (Alkanani, Parrish, Thompson, & Mckenzie, 2007; Freites et al., 2002a, 2002b; Karakoltsidis et al. 1995; King et al., 1990; Orban et al., 2002; Otles & Sengor, 2005; Vernocchi et al., 2007). The ANOVA carried out showed significant differences ($p < 0.05$) in the saturated fatty acids profile among the three origins (data not shown). The highest content of palmitic acid was found in Valencian mussels and the lowest content of stearic acid in samples from the Ebro Delta. This fact entailed an important difference in the total saturated fatty acids proportion between these two sites. These results could be due to the different environment, since some authors have stated that bivalves distributed in organic detritus-rich environments with an abundant bacterial load, has higher proportion of saturated fatty acids (Galap, Netchitaïlo, Leboulenger, & Grillot, 1999).

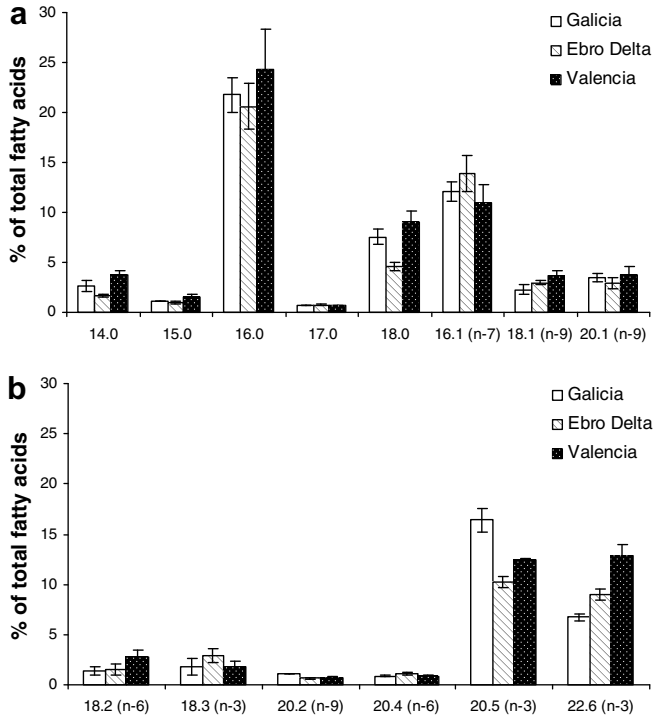

Fig. 1. Percentage of total fatty acids in mussels from Galicia, the Ebro Delta, and Valencia. (a) Saturated and monounsaturated fatty acids; (b) polyunsaturated fatty acids.

Palmitoleic acid (16:1n-7) was the most abundant monounsaturated fatty acid, ranging from 11.02% (Valencia) to 13.83% (Ebro Delta) (Fig. 1a). The same levels were found in the literature (Alkanani et al., 2007; Karakoltsidis et al. 1995; King et al., 1990; Orban et al., 2002; Otles & Sengor, 2005; Vernocchi et al., 2007). There were non significant differences ($p > 0.05$) in the profile of monounsaturated fatty acids among the three origins.

With regard to polyunsaturated fatty acids, eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids were the most important fatty acids in this fraction (Fig. 1b). Galician mussels exhibited a significantly higher amount of 20:5n-3 (16.39%) and lower of 22:6n-3 (6.75%), than mussels from the Ebro Delta and Valencia harbour. Low levels of n-6 (ranging from 2.19% in Galicia to 3.59% in Valencia) and high levels of n-3 polyunsaturated fatty acids (from 22.13% in the Ebro Delta to 27.18% in Valencia), were found in all samples. The intake of n-3 PUFA from natural source may influence the onset and progression of several disease states, including cardiovascular disease and cancer (Shahidi & Miraliakbari, 2004). Ackman (1990) showed the existence of a positive correlation between deaths by coronary illnesses and a high relation of n-6/n-3 (between 12 and 50). In this study, the n-6/n-3 ratio found in all samples was much lower (from 0.09 in Galicia to 0.13 in Valencia) than those cited as harmful in the literature. The PUFA composition also concurs with numerous studies of

mussels and other bivalve species (Alkanani et al., 2007; Freites et al., 2002a, 2002b; Karakoltsidis et al. 1995; King et al., 1990; Orban et al., 2002; Otles & Sengor, 2005; Vernocchi et al., 2007).

3.5. Volatile compounds analysis

Among the volatile compounds identified in this study, 9 were alcohols, 3 ketones, 16 aldehydes, 4 aromatic compounds, 1 furan, and 7 hydrocarbons. Twenty of these compounds were quantified and are shown in Table 4. The other compounds identified were: 1-dodecanol, 1-tridecanol, 1-hexadecanol, 1-pentadecanol, hexanal, heptanal, (*E*)-2-octenal, (*E*,*E*)-2,4-octadienal, decanal, (*E*)-2-decenal, octadecanal, naphthalene, 2-methylnaphthalene, butylated hydroxytoluene, 2-pentylfuran, dodecane, tetradecane, 1-hexadecene, hexadecane, and heptadecane. In general, the compounds identified were common to other studies of volatile fraction of mussels (Cha et al. 1998; Le Guen, Prost, & Demaimay, 2000).

Regarding the long chain alcohols quantified (Table 4), some differences between the three origins were found. Nonanol content was higher in the Valencian samples, and 1-hexanol and 1-octen-3-ol higher in mussels from the Ebro Delta. The highest contents of 1-heptanol and 1-octanol were found in Galician mussels. These differences would not affect the odour of mussels, since alcohols generally do not contribute to the overall flavour because of their high threshold values, unless they are unsaturated (Le Guen et al., 2000).

Whit regard to ketones, significant differences were found in 2-heptanone ($p < 0.05$) and 2-undecanone ($p < 0.001$). The highest concentrations of both compounds were observed in mussels from the Ebro Delta. The differences found in the contents of 2-undecanone would not affect the odour of the products, since Le Guen et al. (2000) reported that this is not an odour-active compound in cooked mussels.

Table 4
Volatile compounds quantified in mussels from Galicia, the Ebro Delta, and Valencia

Volatile compounds[z] (ng/g)	RI	Methods of detection	Origin			α
			Galicia	Ebro Delta	Valencia	
Alcohols						
1-Hexanol	870	RI, MS	41 (26)^{a}	72 (27)^{b}	40 (20)^{a}	**
1-Heptanol	940	RI, MS	52 (13)^{a}	33 (9)^{b}	28 (8)^{b}	**
1-Octen-3-ol	995	RI, MS	55 (13)^{a}	70 (23)^{b}	49 (12)^{a}	*
1-Octanol	1078	RI, MS	36 (7)^{a}	19 (3)^{b}	17 (8)^{b}	**
1-Nonanol	1183	RI, MS	15 (8)^{a}	16 (7)^{a}	41 (27)^{b}	**
Ketones						
2-Heptanone	891	RI, MS	35 (17)^{ab}	49 (26)^{a}	24 (12)^{b}	*
2-Nonanone	1090	RI, MS	32 (10)^{a}	25 (7)^{a}	24 (8)^{a}	ns
2-Undecanone	1295	RI, MS	29 (10)^{a}	44 (5)^{b}	28 (7)^{a}	**
Aldehydes						
(*E*)-2-Hexenal	849	RI, MS	53 (23)^{a}	60 (24)^{a}	52 (23)^{a}	ns
(*Z*)-4-Heptenal	899	RI, MS	50 (10)^{a}	74 (12)^{b}	57 (29)^{a}	**
Benzaldehyde	920	RI, MS	76 (11)^{a}	73 (8)^{a}	56 (13)^{b}	**
Octanal	1011	RI, MS	47 (16)^{a}	49 (12)^{a}	43 (21)^{a}	ns
Nonanal	1108	RI, MS	27 (5)^{a}	30 (3)^{a}	36 (26)^{a}	ns
(*E*,*Z*)-2,6-Nonadienal	1152	RI, MS	37 (42)^{a}	46 (7)^{a}	42 (18)^{a}	ns
(*E*,*E*)-2,4-Decadienal	1325	RI, MS	9 (4)^{a}	16 (3)^{b}	15 (11)^{b}	*
Tridecanal	1595	RI, MS	32 (16)^{a}	37 (11)^{a}	31 (29)^{a}	ns
Hexadecanal	1850	RI, MS	13 (5)^{a}	19 (9)^{b}	16 (6)^{ab}	ns
Aromatics						
Ethylbenzene	862	RI, MS	4 (2)^{a}	7 (4)^{b}	3 (2)^{a}	*
Hydrocarbons						
1-Tetradecene	1489	RI, MS	14 (2)^{a}	11 (4)^{a}	7 (4)^{b}	**
Pentadecane	1586	RI, MS	10 (2)^{ab}	11 (2)^{a}	8 (4)^{b}	ns

Numbers in parentheses represent standard deviation. Means followed by the same letter within the same row are not significantly different, and different letters indicate significant differences. ns, non significant; $^{*}p < 0.05$; $^{**}p < 0.001$.
[z] Mean from three analyses.

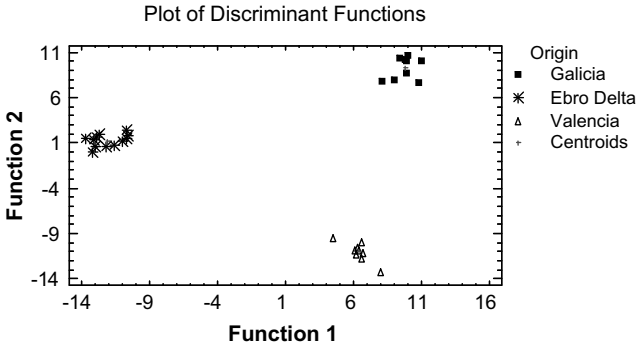

Fig. 2. Discriminant functions plot obtained for the three origins (Galicia, the Ebro Delta, Valencia). Variables: concentrations of volatile compounds.

In general, insignificant differences were found in the aldehydes from the three origins. (*E*,*Z*)-2,6-nonadienal, a compound found in all the samples, plays an important role in the fresh fish odour, due to its low odour threshold (Christian & Grosch, 1996). (*Z*)-4-heptenal and octanal were found in high amounts mainly in mussels from the Ebro Delta. These compounds were reported to be impact odorants in mussel flavour, due to their low detection thresholds (Le Guen et al., 2000).

The presence of aromatic compounds identified in mussels from the three sites, could be due to petroleum contamination, since the uptake of aromatic hydrocarbons has previously been reported in many fish or shellfish (Ogata & Miyake, 1980).

Hydrocarbon concentrations were low in all the samples. These compounds have been reported not to be odour-active components, probably due to their high detection thresholds (Le Guen et al., 2000).

Due to the difficulty in evaluating the behaviour of the volatile fraction when considering each compound individually, the global effect of the origin was analyzed by means of a discriminant analysis. The concentrations of the quantified volatile compounds were the variables and the origin was the factor.

In this analysis two discriminant functions were obtained (*F*1 and *F*2) explaining the 61.39% and 38.61% variance, respectively. Fig. 2 shows the distribution of the three origins in the discriminant space. *F*1 determined the separation of the Ebro Delta samples from the Valencian and Galician samples, while little differences between these latter samples were observed. The three origins were separated according to *F*2. The points belonging to each origin appear concentrated in an area completely separated from the others (Fig. 2). This separation demonstrates that mussels from the three origins showed a characteristic aromatic profile. These results could explain that, in the sensory assessment of these three samples above mentioned, differences in the odour and flavour were found depending on the area of culture (Gómez-Sintes et al., 2004).

4. Conclusions

The origin affected the size as well as the chemical composition of the molluscs. The variations found in mussels depending on the site of culture do not involve important differences in the nutritive value of this product. The differences in the biometric parameters have a direct influence on the aspect of the product that can be decisive for the purchase by the consumers. The variations in the free amino acids contents as well as in the volatile compounds

profile entail differences in the odour, aroma and flavour that were detected in a parallel sensory study to this work. For this reason, the specification of the origin in the label of the fresh product is useful for consumers in order to choose a product according to their preferences. These results provide valuable information for the sector, especially for producers in the Ebro Delta and in Valencia that could apply for the Origin Appellation of their mussels. For this purpose the present work would have to be complemented with the study of other specific parameters.

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