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PII: S0308-8146(14)01130-3
DOI: http://dx.doi.org/10.1016/j.foodchem.2014.07.082
Reference: FOCH 16148

To appear in: *Food Chemistry*

Received Date: 24 April 2014
Revised Date: 14 July 2014
Accepted Date: 15 July 2014

Please cite this article as: Oliveira, A.R., Sykes, A.V., Hachero-Cruzado, I., Azeiteiro, U.M., Esteves, E., A sensory and nutritional comparison of mussels (*Mytilus* sp.) produced in NW Iberia and in the Armona offshore production area (Algarve, Portugal), *Food Chemistry* (2014), doi: http://dx.doi.org/10.1016/j.foodchem.2014.07.082

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Running title: **Sensory and nutritional comparison of *Mytilus* sp. produced in NW and SW Iberia**

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Chemical Compounds studied in this article

Alanine (PubChem CID: 5950), Alpha-linolenic acid (PubChem CID: 5280934), Arachidonic acid (PubChem CID: 444899), Cysteine (PubChem CID: 5862), Docosahexaenoic acid (PubChem CID: 445580), Eicosapentaenoic acid (PubChem CID: 446284), Glycine (PubChem CID: 750), Linoleic acid (PubChem CID: 5280450), Taurine (PubChem CID: 1123), Tyrosine (PubChem CID: 6057).

Keywords: Mussel; *Mytilus* sp.; nutritional composition; offshore aquaculture; sensory analysis.

Abstract

A biometric, nutritional and sensory analysis of raw and cooked mussels comparing *Mytilus* sp. from the north-west coast of Portugal and Spain (Minho and Galicia, respectively) and the new offshore production site of Armona (Algarve, south Portugal) was carried out. In addition, multiple factorial analysis was performed to explore potential relationships between sensory attributes and nutritional content properties between the different mussels. Results showed that, at similar times of sale, biometrics of mussels from Armona and Vigo were similar and bigger than the remaining. Nonetheless, despite some similarities in proximate composition, mussels presented differences in lipid classes, fatty acid content and free amino acids profiles. These differences were not fully reflected in the sensory assessment by the panel, which were able to distinguish different production sites in raw specimens but displayed problems in discrimination these in cooked mussels. Some nutritional components were related to specific sensory sensations.

1. Introduction

The culture of marine molluscs represented 75.5% (13.9 million ton) of world's aquaculture production in 2010, with mussel production reaching approximately 13% (1.8 million ton; FAO, 2014). Mussels' popularity has increased over the past decades due to the presence of bioactive compounds in their meat, which have positive effects on human health (Grienke, Silke & Tasdemir, 2014). Spain is the top producer of mussels (*Mytilus* sp.) in Europe and second worldwide, with a production of nearly 200,000 ton year^{-1} (FAO, 2014). However, the European mussel production has stalled at the end of the XX century due to a reach of the full carrying capacity in traditional locations (Smaal, 2002). This led to an increase in imports by Europe up to nearly 40% of EU production in 2010 (189,700 tons; FAO, 2014) and a loss in revenues for the EU trade balance. Nonetheless, aquaculture production technology has evolved and offshore areas are now being considered as new grounds for production of traditional species.

Portugal does not have a tradition of mussel culture, and its production has been negligible, with relative low commercial demand and value. However, according to Kapetsky, Aguilar-Manjarrez & Jenness (2013), the country has 2,130 km^2 of offshore area with potential for mussel culture due to its hydrographic conditions, wherein the recently established Armona production area in the Algarve is located.

Most of the Spanish mussels' production is carried out in secluded areas, the 'rias'. On the other hand, the lower temperature fluctuations and higher hydrodynamics conditions in the offshore area of Armona (Relvas et al., 2007) favour high food availability as well as a good removal of excretion products. Therefore, different productions sites, with different conditions and culture technologies (rafts in the rias vs. longlines in offshore) should promote changes in the growth and nutritional composition of mussels, which will in turn reflect in their quality as evaluated by consumers. Moreover, mussel's quality is assessed by the consumer as the result of not only its chemical and

biological characteristics, but also its organoleptic properties, such as the appearance of the muscle, the intrinsic flavour and absence of undesirable components (Vernocchi, Maffei, Lanciotti, Suzzi & Gardini, 2007). Together with biometric parameters and chemical composition, sensory characteristics are expected to define the qualities and distinguish mussels produced in different locations (Fuentes, Fernández-Segovia, Escriche & Serra, 2009).

Thus, it makes the more sense to compare mussels from traditional production in Spain with the new offshore production in Portugal. Given this, the main goal of this work was to characterize and compare the biometric parameters (size, weight and meat yield), nutritional content (moisture, ash, total protein and free amino acids, total lipid, lipid class and fatty acids as well as carbohydrates) and sensory aspects (appearance, odour, flavour and texture) of mussels (*Mytilus* sp.) produced in the Armona's Aquaculture Production Pilot Area (APAA) in the Algarve coast (south of Portugal) to mussels from Galicia and North of Portugal.

2. Material and methods

2.1. Samples

Mussels, *Mytilus* sp., from five different locations were studied herein. The offshore (OFF) mussels were cultured in the APAA area (North 37° 01,7692′ N 007° 42,2652′ W; East 37° 00,7677′ N 007° 41,7555′ W; South 36° 59,2953′ N 007° 46,2478′ W; West 37° 00,2960′ N 007° 46,7587′ W), which is located off the Algarve coast (South of Portugal). Individuals were collected in June and July 2011 by the staff of the concessionaire, Companhia de Pescarias do Algarve (Faro, Portugal). Additionally, mussels from 3 sites in Galicia (NW Spain) – unspecified locations in Galicia (SPG), Vigo (VIG) and Pontevedra (PTV) – and from Vila Praia de Âncora, North of Portugal

101 (PTN), were purchased in local markets (Faro, Portugal) between April and July 2011.
102 Mussels from Galicia and North of Portugal were collected 24-48 h before purchase.
103 Samples analysed herein were randomly selected from two 1 kg bags of the same
104 origin/supplier purchased on the sampling day. On the other hand, the offshore mussels
105 were randomly sampled from different longlines 24 h before the assessments. Samples
106 were immediately transported to the laboratory in cooling boxes with ice packs, washed
107 with tap water and stored in a refrigerating chamber at 5±1ºC. Following
108 recommendations in the *Codex Alimentarius* STAN 292-2008 (FAO/WHO, 2008), only
109 mussels without visible damage (e.g. open valves or broken shell) and exceeding the
110 legal/minimum commercial size (50 mm) were analysed herein.
111

112 **2.2. Biometric parameters**

113 Biometric parameters were assessed in a total of 234 specimens (OFF, n = 48; PTN, n =
114 24; PTV, n = 60; SPG = 78; VIG, n = 24). Length (maximum measure along the
115 anterior-posterior axis), width (maximum lateral axis), and height (maximum dorsum-
116 ventral axis) of randomly selected mussels were measured using a digital precision
117 calliper to the nearest 0.1 mm. The animal whole weight (WW) as well as edible
118 fraction (WT) were weighed in a Sartorius U6100 scale (Data Weighing Systems, Inc.,
119 U.S.A.). Meat yield (MY) was calculated as MY = (WW/WT) x 100 (Okumuş &
120 Stirling, 1998).
121

122 **2.3. Nutritional content**

123 Determinations were performed in triplicate using pooled samples. Fifty individuals
124 from each batch/origin were collected and minced in a food processor (Philips HR
125 1396, Royal Philips Electronics, The Netherlands).

126 Fresh samples were collected for moisture and ash determinations, according to the
127 methods described by AOAC (1995), in a Memmert oven (Memmert GmbH & Co. KG,
128 Germany) and a Thermolyne Type 6000 Furnace (Barnestead/Thermolyne Corporation,
129 U.S.A.). The remaining mass was immediately frozen in liquid nitrogen to avoid
130 degradation and later lyophilized before being used in determinations.
131 Total protein was determined according to the Kjeldahl method (AOAC, 1995), with a
132 conversion factor of 6.25. Samples were digested in a Gerhardt Kjeldatherm and
133 distilled in a Gerhardt Vapodest 1 (C. Gerhardt GmbH & Co. KG, Germany). Free
134 amino acids (FAA) were extracted with 0.1M hydrochloric acid (HCl) and the
135 homogenate was centrifuged by ultrafiltration (10kDa, 2500g, 20 min, 4ºC).
136 Derivatization using phenylisothiocyanate (PITC) was conducted according to the
137 PicoTag™ method described by Cohen, Meys and Tarvin (1989). The derivatized
138 amino acids and standard solutions were analysed by reverse-phase high pressure liquid
139 chromatography (HPLC-RP) in a Waters™ LC system with a PicoTag™ column (3.9 x
140 300 mm), a column heater (at 46ºC), two pumps, an auto-sampler and a variable
141 wavelength UV/VIS detector, according to the conditions described by Cohen et al.
142 (1989). The chromatograms were monitored at a wavelength of 254 nm. Identification
143 and quantification of the peaks were carried out with the Breeze software (Waters
144 Corp., U.S.A.). Amino acid standard solutions with the internal standard (norleucine)
145 were prepared and derivatized following the same procedure described for the samples.
146 Total carbohydrates were determined according to the method described by Dubois,
147 Gilles, Hamilton, Rebers & Smith. (1956). Sample readings were performed in a
148 Hitachi U-2000 spectrophotometer, at 490nm.
149 Total lipid (TL) was extracted with chloroform:methanol (2:1 v/v) containing 0.01% of
150 butylatedhydroxytoluene (BHT) as antioxidant (Christie, 1982). Lipid classes (LC) and

fatty acids (FA) were determined at IFAPA – Agua del Pino (Huelva, Spain). Total lipid samples were separated into classes by one-dimensional double-development high-performance thin-layer chromatography (HPTLC) using methyl acetate/ isopropanol/ chloroform/ methanol/ 0.25% (w/v) potassium chloride (KCl; 25:25:25:10:9 by vol.), as the polar solvent system and hexane/diethyl ether/glacial acetic acid (80:20:2 by vol.), as the neutral solvent system. Lipid classes were quantified by charring with a copper acetate reagent followed by calibrated scanning densitometry using a CAMAG TLC Scanner 3 dual wavelength flying spot scanner (Mutten, Switzerland) dual wavelength flying spot scanner (Olsen & Henderson, 1989). Total lipid extracts were subjected to acid-catalysed transmethylation for 16 h at 50ºC, using 1mL of toluene and 2 mL of 1% sulphuric acid (v/v) in methanol. The resulting fatty-acid methyl esters (FAME) were purified by thin-layer chromatography (TLC), and visualized with iodine in chloroform:methanol (2:1 v/v) 98% (v/v) containing 0.01% BHT (Christie, 1982). Prior to transmethylation, heneicosanoic acid (21:0) was added to the TL as an internal standard. FAME were separated and quantified using a SHIMADZU GC 2010 (Kyoto, Japan) gas chromatograph equipped with a flame-ionisation detector (250ºC) and a fused silica capillary column Tecnokroma — Suprawax-280TM (15 m × 0.1 mm I.D.). Helium was used as a carrier gas and the initial oven temperature was 150ºC, followed by an increase at a rate of 30ºC min-1 to a final temperature of 250ºC for 7 min. Individual FAME were identified by reference to authentic standards and to a well-characterized fish oil.

BHT, KCl, potassium bicarbonate, and iodine were supplied by SIGMA CHEMICAL Co (St. Louis, USA). TLC (20x20 cm x 0.25 mm) and HPTLC (10x10 cm x 0.15 mm) plates, pre-coated with silica gel (without fluorescent indicator) were purchased from MACHEREN-NAGEL (Düren, Germany). All organic solvents used for gas

chromatography (GC) were of reagent grade and were purchased from PANREAC (Barcelona, Spain).

2.4. Sensory analysis

All sensory analysis sessions were performed according to ISO standards (ISO 2001, 2008) in a sensory analysis room (in the Department of Food Engineering, DEA-ISE, University of the Algarve) compliant with ISO (2007), by a panel of 12 people co-opted from the staff of DEA-ISE with previous experience in sensory analysis of food products. Nonetheless, in order to familiarize the panel with the sensory assessment of mussels and to optimize the tables used for sensory evaluation, five training sessions were conducted. Initially, considering the specific characteristics to be assessed (FAO/WHO, 2001), panellists freely used terms from a pre-determined vocabulary set (Gökoglu, 2002). Results were used to elaborate a preliminary version of the tables for sensory evaluation based on Torry Sensory Assessment schemes (Archer, 2010). These tables were optimized in terms of descriptors and assessment criteria during the following training sessions.

The sensory analysis comprised fresh and cooked mussel samples. The sensory attributes evaluated, using a 0-5 point category scales, were: a) odour, muscle/meat appearance and texture for fresh mussel; and b) odour, flavour and texture for cooked mussel, as shown in Table I. Twenty four individual mussels were randomly selected from each batch of different origin and kept on ice until assessment. Two mussels (one fresh and one cooked) of each batch were presented sequentially to each panellist in 7x7x2 cm white, equal-sized dishes, properly coded. Fresh mussels were shucked immediately before testing while the cooked mussels were steamed at 400W in a Moulinex FM 2535 microwave (Moulinex, France) for 1.5 min without seasoning.

201

202 **2.5. Data analysis**

203 Results are reported as means ± standard deviation or estimates ± standard error (where
204 appropriate). The significance level was set at 5%.

205 The relationship among length, width, height and weight variables was analysed
206 through multiple linear regression.

207 Differences in biochemical compositions of mussels originated from distinct locales
208 where tested using one-way ANOVA per parameter. Values expressed as relative
209 percentage were arc-sine square-root transformed prior to analysis. Significant
210 differences in ANOVA were further studied using Fisher's least significant difference
211 (LSD) post-hoc test. Whenever homogeneity of variances could not be met (viz. FAA,
212 LC and FA), Welch ANOVA and the Games-Howell post-hoc test were used instead.
213 IBM® SPSS® Statistics 19 (IBM® Co., USA) was used in all the previous statistical
214 calculations.

215 Sensory panel performance was assessed using three-way ANOVA per parameter and
216 considering the distinct origins (factor Product) and session-to-session differences
217 (factor Session) in panellists' results (factor Panellist). At this stage, data pertaining to
218 mussels from PTN and VIG were excluded since they were analysed once. The
219 interactions of factors Product×Panellist and Panellist×Session were used to assess
220 panellists' discriminating power and consistency, respectively. A multivariable
221 principal component analysis (PCA)-based approach was used to compare mussels'
222 sensory profiles (Husson, Lê & Pagès, 2010). The descriptors/sensory attributes that in
223 the initial ANOVA were found not statistically significant i.e. $p>0.05$ were not
224 considered herein. Results were augmented via bootstrap (R=500), that allowed the
225 estimation of 95% confidence ellipses around products' average points. Finally,

products were compared using T^2 Hotelling test. The interest of implementing the PCA on these data was assessed using Bartlett's sphericity test and Keiser-Mayer-Olkin measure of sampling adequacy (KMO MSA). The procedures described above were carried out for fresh and cooked mussels' results of sensory analysis using the package SensomineR (Lê & Husson, 2008) for the R software version 2.14.0.

A multiple factorial analysis (MFA) was carried out, using the package FactoMineR for the R software version 2.14.0 (Husson, Lê & Pagès, 2010), to explore the potential relations between sensory attributes and physical-chemical properties among the distinct mussels (PTN, OFF and VIG). The MFA, derived from PCA and canonical correlation analysis (CCA), was carried out using average data for odour, flavour and texture parameters of cooked mussels and the corresponding averages of the most relevant FAA and FA (viz. volatile essential amino acids and fatty acids that were found significantly different between mussel batches).

3. Results

3.1. Biometric data

Differences were found in all the parameters being assessed, except for the meat yield. In general, the PTV and SPG mussels were smaller and lighter than mussels from the remaining batches. Regarding length, VIG presented the larger individuals (83.13 ± 1.29 mm) followed by OFF mussels. Both OFF and VIG presented the highest width, height and weight, while SPG and PTV included the individuals with the smallest measurements, respectively ($p<0.05$). Interestingly, OFF and VIG mussels were quite similar in size and weight. No significant correlations were found between length and width versus weight ($p>0.01$). However, height was found to be significantly correlated to weight ($p<0.01$). No significant differences ($p>0.05$) were found in MY between OFF

and PTN mussels in spite of the differences found in shell morphology.

3.2. Nutritional content

The proximal composition of the edible portion of PTN, OFF and VIG mussels is presented in table II. Mussels from these 3 locations showed different proximal composition. Moisture and ash were higher ($p<0.05$) in PTN mussels. PTN and VIG mussels presented the higher content in carbohydrates (28 and 32%, respectively; table II). No significant differences ($p<0.05$) regarding protein and lipid content were found between mussels.

As for LC, PTN mussels displayed the highest value of polar lipids, while no differences ($p>0.05$) were found regarding neutral lipids between all the sites. This was due to the slightly higher content in phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidyl-ethanolamine (PE) measured in PTN mussels ($p<0.05$; Table II). The biggest differences between production sites were observed in the neutral lipids classes, where PTN mussels and VIG displayed the highest cholesterol (CHO) content ($p<0.05$). On the other hand, the OFF mussels displayed the highest ($p<0.05$) content in triglycerides (TG) and FA.

Of the 56 FA identified, palmitic acid (16:0), stearic acid (18:0), dimethyl acetal stearic acid (DMA 18:0), palmitoleic acid (16:1n7), eicosapentaenoic acid (EPA; 20:5*n*3), and docosahexaenoic acid (DHA; 22:6*n*3) totalized around 70% of the total FA content (Table III). No significant differences ($p>0.05$) were observed regarding the sum of polyunsaturated fatty acids (PUFA) between sites. However, the sum of saturated fatty acids (SFA) was higher in PTN and OFF ($p<0.05$) and a higher content in monounsaturated fatty acids (MUFA) was observed in VIG mussels ($p<0.05$). It is also interesting that the highest values of the PUFAs *n*6 group were composed by

arachidonic acid (ARA; 20:4*n*6) and linoleic acid (LA; 18:2*n*6), both in the VIG mussels ($p<0.05$). VIG specimens displayed the highest content in EPA, while OFF mussels had the highest content in DHA ($p<0.05$).

On the other hand, MUFA displayed the lowest content in all the mussels analysed and was mainly composed by palmitoleic acid (16:1*n*7), being higher in VIG mussels ($p<0.05$).

As regards the FAA content, differences ($p<0.05$) were noted between the three production sites. The highest content in total essential amino acids was observed in the VIG mussels, while both OFF and VIG specimens displayed similar but higher values of total non-essential amino acids respect to PTN (Table IV). Lysine was the most abundant essential amino acid found in mussels from all production sites. As for non-essential amino acids, taurine was the most abundant, displaying the highest content in VIG mussels (Table IV). Besides taurine, FAA profiles were rich (in decreasing order) in glycine, alanine, glutamic acid and arginine. The OFF mussels presented the lowest values of taurine, alanine and glutamic acid of the analysed locales, but its glycine content more than doubled (1648.65 µmol g-1 DW) that of the remaining mussels ($p<0.05$; Table IV). Differences were also registered for leucine, valine, phenylalanine, tyrosine asparagine and ornithine contents between the 3 different origins ($p<0.05$).

3.3. Performance of the sensory analysis panel

Globally, panellists' performance during and between sensory analysis sessions was good, i.e. stable and consistent. Regarding fresh and cooked mussels, 6 and 7 out of 10 panellists, respectively, were able to discriminate the mussels based on several attributes. There were, however, a few discrepancies in the evaluation of some of the attributes by some panellists. Although there were significant differences among

panellists, these were not seen in the evaluation of the attributes between sessions ($p>0.09$). Taking the session factor into consideration, the panellists were highly consistent in the evaluation of mussels throughout the sessions (repeatability was observed in ca. 93% of the assessments in both fresh and cooked mussels).

The attributes "orange colour" (ORCL), "moist appearance" (MOAP) and "firmness" (FIRM) were the ones where panellists most disagreed in fresh mussels' assessments (up to 21% of the individual assessments did not compare to the whole panel). In addition, colour was one of the sensory analysis attributes that, in the present study, obtained less agreement and discriminating power by the panellists, during fresh mussel sensory analysis. As for cooked mussels, the agreement between individual panellist assessment and the panel was lower (≈40%).

3.4. Sensory analysis of fresh mussels

In a multidimensional perspective, bootstrap-augmented PCA helped summarizing the information between variables in two orthogonal components, which explained more than 93% of the total variance of the original variables: the 1st component (PC1) with 83.54% of the overall inertia and the 2nd component (PC2) with 10.12%. According to both Bartlett's test ($\chi^2 = 526.17$; $p<10^{-6}$) and KMO MSA (0.7720), PCA was deemed efficient. The PC1 dimension was mainly defined by appearance and odours (positive PC1 dimension) in contrast to firmness (negative PC1 dimension). The main descriptors defining PC2 dimension were those related to texture, firmness (positive PC2 dimension) and, to lesser extent, elasticity (negative PC2 dimension).

Despite the five training sessions, panellists had difficulty in evaluating some attributes, namely "firmness", "consistency" or "juiciness" (Fig. 1A), which are used to describe texture. Still regarding the PCA plot, confidence ellipses allowed distinguishing OFF,

326 SPG and VIG mussels from PTV and PTN mussels (Fig. 1C). These two "groups" were
327 well differentiated using the PC1, wherein attributes related to appearance and odour
328 were located on the positive PC1 and strongly correlated to each other. The PC2,
329 defined mostly by firmness (positive coordinate) and by elasticity (negative coordinate),
330 further discriminated SPG and VIG mussels, both produced in Galicia, and, to a lesser
331 extent, mussels from the Algarve (OFF). The Hotelling test confirmed significant
332 differences ($p<0.05$) between all mussels except those from PTN and PTV.
333
334 **3.5. Sensory analysis of cooked mussels**
335 Colour, glossiness and appearance of tissues' surfaces of cooked samples were clearly
336 altered during steaming. It was interesting to verify that OFF mussels were not readily
337 distinguished from the other mussels' production sites in terms of sensory attributes. In
338 addition, cooked OFF mussels' were clearly described by the panellists as more
339 succulent and with the best characteristic flavour, followed by VIG specimens.
340 The first and second components of PCA (Fig. 1B) explained more than 96% of the
341 total variance (85.03% for PC1 and 11.06% for PC2). However, since PC2 displayed an
342 eigenvalue <1, PC1 solely could have been retained for interpretation. According to
343 both Bartlett's test (χ^2 = 396.9; $p<10^{-6}$) and KMO MSA (0.7215), PCA was judged
344 efficient.
345 Only five sensory attributes effectively explained the majority of the differences
346 between cooked mussels: fresh (FROD) and intrinsic odours (INTOD), characteristic
347 flavour (CHFLV), succulence (SUCC) and smoothness (SMO). SMO showed
348 comparatively high loadings on the positive dimension of both PC1 and PC2 (fig. 1B),
349 whereas the remaining attributes (particularly SUCC and CHFLV) had strong, positive
350 loadings on the PC1. The overlapping confidence ellipses presented in figure 1D

showed a less clear discrimination of production sites using cooked mussels' data. The retained sensory attributes characterized mussels from SPG and OFF has having pronounced CHFLV and SUCC, FROD and INTOD, and being perceived as smooth in sharp contrast to VIG, PTV and PTN mussels. The Hotelling test confirmed the significant differences ($p<0.05$) in sensory profiles between the OFF mussels and the ones from PTV and VIG, as well as between the SPG mussel and the ones from PTN and PTV. On the other hand, no differences were found between the OFF and SPG mussels ($p=0.324$).

3.6. Combining sensory and nutritional content of cooked mussels

MFA, a PCA-based methodology on the merged (sensory and instrumental variables) data, enriched the interpretation of the sensory data by showing how the physical-chemical properties are reflected by specific sensations. In this study, the 18:0 SFA appeared to be related to the fresh odour attribute, and the DHA/EPA ratio related to the seaweedy odour. The FA 16:0 and DHA also appeared to contribute to the characteristic flavour of mussel (Fig. 2). The FAA were greatly correlated to the firmness of mussel's meat (Fig. 2), particularly alanine (Ala), cysteine (Cys), taurine (Tau) and tyrosine (Tyr). In addition, glycine was closely related to the smoothness (SMO) and toughness (TOUGH).

4. Discussion

OFF and VIG mussels were quite similar in length, width and height to mussels from Galicia and the Ebro Delta, characterized by Fuentes et al. (2009), which were generally bigger than those from Valencia. As for MY, mussels from OFF and PTN probably had higher content than any of the mussels of the previous study. On the other hand, OFF

and PTN mussels displayed higher MY than those of the Adriatic Sea (25.2%; Vernocchi et al., 2007). The differences found between different samples and results found in literature are easily justified by culture density-dependent effects (Cubillo, Peteiro, Fernández-Reiriz & Labarta, 2012), temperature and season (Bayne & Worrall, 1980; Okumuş & Stirling, 1998), availability of food (e.g. phytoplankton blooms) and spawning condition (Strohmeier, Duinker, Strand & Aure, 2008), etc. As a matter of fact, MY depends on complex interactions including not only temperature and salinity but, more importantly, food supply and gametogenic cycle (Okumuş & Stirling, 1998). However, there is no way to reliably obtain data on sex nor precise the maturity stage of mussels based on methods such as mantle colours observation, condition indices and meat yield. This is due to the fact that the reproductive cycle varies considerably between species and with geographical locations (Gabbott, 1976). Nevertheless, the samples were available to the customer at similar times so a comparison of products is justified and was established.

Proximate composition of mussels from three sampled locations (PTN, VIG and OFF) only showed differences in moisture, ash and carbohydrates. Since the technology of culture was similar (longlines/hanging ropes), the relatively low values of carbohydrates and the marginal differences in ash observed in the OFF mussels were most probably due to the different hydrodynamic conditions of this offshore culture area, which will interfere with mussel metabolism in a set of complex interactions between temperature, food availability, growth and reproduction cycle (Gabbott, 1976). The reproductive cycle of mussels in Galicia does not necessarily follow patterns described for other regions, since there are differences among mussel populations of different geographical areas, among populations from close locations and interannual differences at the same location (Villalba, 1995). According to data from Relvas et al., (2007), all the mussel

401 production sites of samples used in the present study display upwelling, which promotes
402 phytoplankton blooms, but its temperature profiles are different throughout the year. In
403 fact, the temperature profile of the Armona site is characterized by higher seawater
404 temperatures when compared to those of NW of the Iberian Peninsula, which might
405 promote faster growth and possibly two peaks of reproduction (one in spring and
406 another in summer), as reported by Villalba (1995) to sometimes occur in Vigo.
407 Moreover, temperature will also affect the composition and availability of food and/or
408 consequently the timing and duration of the reproductive cycle and number of
409 spawnings per year (Gabbott, 1976), which will affect the nutritional content of
410 mussels. For instance, mussels (*M. galloprovincialis*) from the Adriatic Sea, sampled at
411 similar months, showed higher protein levels (between 46.98 and 52.66%), but lower
412 lipids, ash and MY content (5.6-8.1%, 12.8-13.8% and 13.4-21%, respectively;
413 Vernocchi et al., 2007), than those of OFF.
414 Moreover, the variations observed in the levels of total lipids, neutral lipids and fatty
415 acids in mussels in the present study should be related to the nature of their local diet,
416 which depends on the conditions already enumerated above. The samples showed a FA
417 profile rich in both SFA and PUFA, which means that all the locations were probably
418 rich in detritus, bacteria, nanozooplankton and phytoplankton (Freites, Labarta &
419 Fernández-Reiriz, 2002b). Nonetheless, typically mussels from Galicia (NW Spain)
420 display higher levels of EPA when compared to those from the warmers waters of the
421 Mediterranean (e.g. Valencia or Ebro delta), which in turn display higher DHA content
422 and a DHA/EPA ratio near 1 (Fuentes et al., 2009), similar to what was observed for the
423 OFF mussels. The higher percentage of EPA, ARA and 18:1n7 and lower percentage of
424 DHA and DHA/EPA ratios verified in the VIG mussels might be related to the higher
425 diatom content which is normally verified in estuarine areas, such as the Vigo ria.

Still, it needs to be considered that in the present study PTN and VIG mussels were depurated prior to being marketed, which most probably interfered with their nutritional profile. While OFF mussels are cultured in a class A area, the remaining specimens are grown in class B areas and are, therefore, subjected to depuration in order to reduce faecal bacterial contamination. During depuration, shellfish are fasted, which results in excretion of waste products of metabolism (Lee, Lovatelli & Ababouch, 2008), and forced to expend their energy reserves in their metabolic processes. This will influence their nutritional quality and organoleptic characteristics (Ruano, Ramos, Quaresma, Bandarra & Fonseca, 2012). In fact, the VIG mussels displayed lower TG and higher FA than those of Freites, Fernández-Reiriz & Labarta (2002a), which were collected in a nearby geographical location (ria Arosa) but not subjected to depuration.

The FAA profiles of VIG were similar to those reported by Fuentes et al. (2009), with a higher taurine content followed, in decreasing order, by arginine, glycine, and alanine. Taurine plays an important role in human physiology (Huxtable, 1992) but no important effect on the formation of aroma active components (Fuke, 1994). On the other hand, the glycine value registered in the OFF mussels was extremely high, reaching values similar to those of taurine, which were not registered by Fuentes et al. (2009) in any geographical location of the Iberian Peninsula. Differences in the contents of some of the FAA, e.g. Leucine, Valine, Phenylalanine, Tyrosine, Asparagine or Ornithine, among locations can be attributed to different environmental and feeding conditions of production areas as pointed out in other studies (Fernández-Reiriz *et al*., 1996; Orban *et al*., 2002; Fuentes *et al*., 2009). Moreover, differences in total FAA could in part be caused by proteolysis that might have occurred to a lesser extent in the samples from offshore area due to the shorter time from harvesting at origin to their arrival at the laboratory as proposed by Fuentes *et al*. (2009).

Results show that there were discrepancies in the assessment of some of the attributes by some panellists, either in fresh or cooked mussels. In spite of Caglak, Cakli & Kilinc (2008) suggesting that a numeric acceptability scale from 0 to 5 points was suitable to evaluate fresh and cooked mussels, the lack of coherence in the assessment of some of the attributes observed herein may reflect some disagreement of the panellists regarding the use of the acceptability scale (Esteves, 2008). While the evaluation of "moist appearance" and "firmness" is directly related to panel sensory ability, the differences in the assessment of "orange colour" in fresh mussels has a biological explanation since, in this species, gonad coloration varies greatly between individuals (Mikhailov, Mario & Mendez, 1995). Therefore, individual discrepancies of the panel might extend beyond sensory assessment and be related to biological factors. As for the difficulty in the assessment of "firmness", "consistency" or "juiciness", these are probably due to the fact that, according to Costell & Durán (2005), food texture is the result of different natures' stimuli, and its assessment is a dynamic and complex process that implies visual perception of the products, their response to handling and the integration of the sensations experienced in the mouth during chewing and swallowing.

As in a previous study by Gómez-Sintes, Fuentes, Fernández-Segovia, Serra & Escriche (2004), panellists were not able to find any differences between appearance and colour of cooked mussels; albeit, the heat treatment to which samples are subjected should have a minimum impact on their innate characteristics (Hyldig (2010). On the other hand, the heat treatment allows the release of volatile compounds that enhance flavours (Ólafsdóttir & Jónsdóttir, 2010) and herein contributed to the distinction between mussels in terms of CHFLV, FROD and INTOD.

It was interesting to verify that OFF mussels were not readily distinguished from the other mussels' production sites in terms of sensory attributes. It was expected that the

476 lack of depuration in OFF mussels influenced the perception of sensory attributes due to
477 already explained differences in terms of nutritional content.
478 The nutritional content was reflected in the sensory perception of mussels' quality
479 characteristics. For instance, the lipid conversions (mainly PUFA) into volatile
480 compounds resulted in the variation of the specific characteristics of flavour, as
481 described by Ólafsdóttir & Jónsdóttir (2010) for other species. Fuentes et al. (2009)
482 linked the high concentration of FAA found in mussels with the perception of intense
483 odour and flavour attributes: aspartic acid (acidity), glutamic acid (flavour intensifier),
484 arginine (bitterness), glycine and alanine (sweetness). Surprisingly, most panellists in
485 this study had trouble evaluating sweetness, but this attribute could be subtly expressed
486 in the salty/characteristic flavour of cooked mussel. In fact, the essential amino acids of
487 ramified chain (valine, isoleucine and leucine), the ones containing sulphur (methionine
488 and cysteine) and the aromatics (phenylalanine and tyrosine) are the most important
489 amino acids contributing to odour and flavour (Aristoy & Toldrá, 2010).
490

491 **5. Conclusions**

492 The production site influenced the size and nutritional content of mussels. As for the
493 sensory analysis, panellists were able to distinguish mussels of different origins to some
494 extent. Flavour was the distinguishing characteristic that panellists used to favour OFF
495 mussels. From a marketing point of view, both biochemical and sensory characteristics
496 ensure that the offshore mussel produced in the Algarve coast (OFF) will have good
497 acceptability by the final consumer, and will surely be able to compete with other
498 mussels currently found in the market, namely the mussels produced in the Galician rias
499 (Vigo, Arousa and others), seafood product that is registered in the EU as a Protected
500 Designation of Origin (PDO).

Acknowledgements

A.V. Sykes wishes to thank Fundação para a Ciência e a Tecnologia (FCT) for his post-doctoral grant (SFRH/BPD/36100/2007). Ismael Hachero-Cruzado's post-doc contract is supported by INIA. This work was funded by Project SEPIAMETA (PTDC/MAR/102348/2008) granted by FCT. Lipid class and fatty acid determinations were funded by project INTERREG 0251_ECOAQUA_5_E.

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630

631 List of Figures

632

633 Figure 1 – (top) Principal component analysis (PCA) of the attributes (variables) and
634 individual quotas in (A) fresh and (B) cooked mussels' assessment. Coloured dots
635 correspond to the bootstrap-generated, virtual panel; arrow directions indicate the
636 importance by principal component; dots of the same colour show consensus in the
637 evaluation. Legend: CHFLV - characteristic flavour; ELAS - elasticity; FIRM -
638 firmness; FROD - fresh odour; INTOD - intrinsic odour; MOAP - moist appearance;
639 ORCL - orange colour; SEAWOD - seaweedy odour; SHAP - shiny appearance; SMO -
640 smoothness; SRFAP - surface appearance; SUCC - succulence; Dim 1 - dimension or
641 principal component 1; Dim 2 - dimension or principal component 2. (bottom)
642 Multidimensional PCA of (C) fresh and (D) cooked mussels. Ellipses represent the 95%

643 confidence intervals estimated via bootstrap (500 iterations), wherein the central points
644 correspond to the average by batch. Legend: OFF - offshore; PTN - North of Portugal;
645 PTV - Pontevedra; SPG - Galicia; VIG – Vigo.
646
647 Figure 2 - Biplot of the two principal components resulting from the multifactorial
648 analysis (MFA), considering the relevant variables in the sensory and biochemical
649 analysis, of mussels from the different origins studied. Legend: Sens. - sensory
650 attributes; CHEW - chewiness; CHFLV - characteristic flavour; CONS - consistency;
651 FIRM - firmness; FROD - fresh odour; INTOD - intrinsic odour; SAFLV - salty
652 flavour; SEAWOD - seaweedy odour; SMO - smoothness; SUCC - succulence;
653 SWFLV - sweet flavour; TOUGH - toughness. FFA - free fatty acids; ALA - alpha-
654 linolenic acid; ARA - arachidonic acid; C16.0 - saturated C16:0 fatty acid; C18.0 -
655 saturated C18:0 fatty acid; DHA - docosahexaenoic acid; DHA.EPA - DHA/EPA ratio;
656 EPA - eicosapentaenoic acid; EPA.ARA - EPA/ARA ratio; LOA - linoleic acid; n3.n6 -
657 omega-3/omega-6 fatty acids ratio. AA - aminoacids; Ala - Alanine; Cys - Cystein; Glu
658 - Glutamic Acid; Gly - Glycine; Ile - Isoleucine; Leu - Leucine; Met - Methionine; Phe -
659 Phenylalanine; Tau - Taurine; Tyr - Tyrosine; Val - Valine. Dim 1 - dimension or
660 principal component 1; Dim 2 - dimension or principal component 2.

Table I – Attributes, terms/descriptors and scores optimized for sensory analysis of fresh and cooked mussel.

Mussels/Attributes		**Score/Descriptors**
Fresh mussels		
Odour	Fresh	0-Absent to 5-Intense
	Intrinsic/Characteristic	0-Absent to 5-Intense
	Marine/Seaweed	0-Absent to 5-Intense
Muscle/Meat appearance	Brightness	0-Absent to 5-Intense
	Moisture	0-Absent to 5-Intense
	Orange colour	0-Pale to 5-Bright
	Surface	0-Rough to 5-Smooth
Texture	Firmness	0-Firm to 5-Tender
	Consistency	0-Tough to 5-Soft
	Elasticity	0-Rigid to 5-Elastic
	Smoothness	0-Grainy to 5-Smooth
Cooked mussels		
Odour	Fresh	0-Absent to 5-Intense
	Intrinsic/Characteristic	0-Absent to 5-Intense
	Marine/Seaweed	0-Absent to 5-Intense
Flavour	Intrinsic / Characteristic	0-Absent to 5-Intense
	Salty	0-Absent to 5-Intense
	Sweet	0-Absent to 5-Intense
Texture	Firmness	0-Firm to 5-Tender
	Consistency	0-Resistant to 5-Fragile
	Toughness	0-Tough to 5-Soft
	Chewiness	0-Hard to 5-Easy
	Juiciness	0-Dry to 5-Juicy
	Smoothness	0-Grainy to 5-Smooth

Table II - Proximal composition and lipid classes profiles of North Portugal (PTN), Offshore (OFF) and Vigo (VIG) mussels.

Proximal Composition	PTN	OFF	VIG
Moisture*	87.59 ± 0.27^{c}	83.94 ± 0.27^{b}	81.71 ± 0.31^{a}
Ash	23.22 ± 0.54^{b}	16.41 ± 0.40^{a}	15.29 ± 0.14^{a}
Total Protein*	39.17 ± 2.99	42.94 ± 2.30	37.85 ± 0.86
Total Carbohydrates*	27.71 ± 1.00^{b}	20.37 ± 0.69^{a}	31.93 ± 2.37^{b}
Total Lipids	10.54 ± 1.04	11.71 ± 0.74	9.09 ± 0.88
Lipid Classes			
Lysophosphatidylcholine (LPC)	0.37 ± 0.13^{ab}	0.58 ± 0.08^{b}	0.31 ± 0.05^{a}
Lysophosphatidylethanolamine (LPE)	0.84 ± 0.21^{b**}	0.81 ± 0.26^{b}	0.00 ± 0.00^{a}
Phosphatidylcholine (PC)	12.14 ± 0.37^{b}	10.70 ± 0.30^{a}	10.50 ± 0.20^{a}
Phosphatidylserine (PS)	11.13 ± 0.82^{b}	7.90 ± 1.04^{a}	8.56 ± 0.50^{a}
Phosphatidylinositol (PI)	3.19 ± 0.26^{b}	3.21 ± 0.35^{b}	1.97 ± 0.24^{a}
Phosphatidylethanolamine (PE)	12.79 ± 0.38^{b}	11.10 ± 0.53^{a}	10.77 ± 0.16^{a}
Diacylglycerol (DAG)	1.10 ± 0.38^{a}	1.47 ± 0.06^{b}	1.64 ± 0.19^{b}
Cholesterol (CHO)	18.34 ± 1.67^{b}	13.31 ± 0.65^{a}	15.84 ± 0.61^{b}
Free Fatty Acids (FFA)	11.84 ± 1.55^{b}	14.55 ± 1.09^{c}	6.85 ± 0.70^{a}
Triglycerides (TG)	15.20 ± 0.49^{a}	21.99 ± 1.21^{b}	31.46 ± 0.73^{c}
Sterol Esters + Waxes (SE+WE)	5.70 ± 0.27^{a}	8.13 ± 0.80^{b}	5.78 ± 0.02^{a}
Pigments (Pigm)	8.27 ± 0.29^{c}	6.65 ± 0.35^{b}	5.92 ± 0.05^{a}
Polar Lipids	40.17 ± 2.14^{b}	34.29 ± 2.44^{a}	32.11 ± 0.74^{a}
Neutral Lipids	60.45 ± 3.66	66.10 ± 3.60	67.49 ± 1.95

Proximal composition values are expressed in % DW, except moisture. Lipid classes are expressed in relative percentage of total lipids (equivalent to g.100g^{-1} DW). Samples for proximal composition n=3. Samples of PTN and OFF for lipid classes n=3; for VIG n=2. Samples signalled with ** correspond to n=2 by removal of outlier. Different letters indicate significant differences for $p<0.05$ (LSD post-hoc test; * Games-Howell post-hoc test).

Table III - Free fatty acids profiles of North Portugal (PTN), Offshore (OFF) and Vigo (VIG) mussels.

Free Fatty Acids (% Lipids)	PTN	OFF	VIG
14:0	2.07 ± 0.11[a]	3.12 ± 0.08[b]	2.88 ± 0.11[b]
16:0*	24.23 ± 0.50[b]	25.71 ± 0.44[b]	21.89 ± 0.85[a]
18:0	7.84 ± 0.32[c]	6.61 ± 0.24[b]	5.92 ± 0.14[a]
18:0 DMA	6.48 ± 0.24[b]	4.74 ± 0.56[a]	4.61 ± 0.52[a]
16:1*n*7	3.22 ± 0.07[a]	3.30 ± 0.10[a]	5.88 ± 0.08[b]
18:1*n*9	1.66 ± 0.11[a]	1.63 ± 0.04[a]	1.89 ± 0.07[b]
18:1*n*7	1.70 ± 0.03[b]	1.57 ± 0.04[a]	2.12 ± 0.00[c]
18:2*n*6 (LA)*	1.53 ± 0.01[a]	1.54 ± 0.02[a]	1.80 ± 0.03[b]
18:3*n*3 (ALA)*	1.10 ± 0.00[a]	1.61 ± 0.01[b]	1.40 ± 0.05[ab]
18:4*n*3	1.46 ± 0.03[a]	2.52 ± 0.03[c]	1.87 ± 0.07[b]
20:1*n*9	2.12 ± 0.07[b]	2.02 ± 0.08[b]	1.81 ± 0.01[a]
22:1*n*9	3.28 ± 0.66[b]	2.10 ± 0.18[a]	2.85 ± 0.15[ab]
20:4*n*6 (ARA)	1.92 ± 0.05[b]	1.52 ± 0.06[a]	2.46 ± 0.08[c]
20:5*n*3 (EPA)	8.87 ± 0.11[a]	11.70 ± 0.21[b]	16.10 ± 0.68[c]
22:6*n*3 (DHA)	12.38 ± 0.15[b]	14.60 ± 0.52[c]	8.39 ± 0.28[a]
UK	9.05 ± 0.49[b]	6.35 ± 0.40[a]	8.35 ± 0.69[b]
Σ SFA	43.61 ± 0.50[b]	42.83 ± 1.00[b]	37.49 ± 0.73[a]
Σ MUFA	15.35 ± 0.55[b]	13.20 ± 0.12[a]	17.27 ± 0.26[c]
Σ PUFA*	41.04 ± 0.09	43.97 ± 1.10	45.24 ± 0.99
*n*3/*n*6	5.17 ± 0.10[a]	7.41 ± 0.02[c]	5.48 ± 0.03[b]
DHA/EPA	1.39 ± 0.00[c]	1.25 ± 0.03[b]	0.52 ± 0.00[a]
EPA/ARA	4.63 ± 0.12[a]	7.71 ± 0.15[c]	6.54 ± 0.05[b]

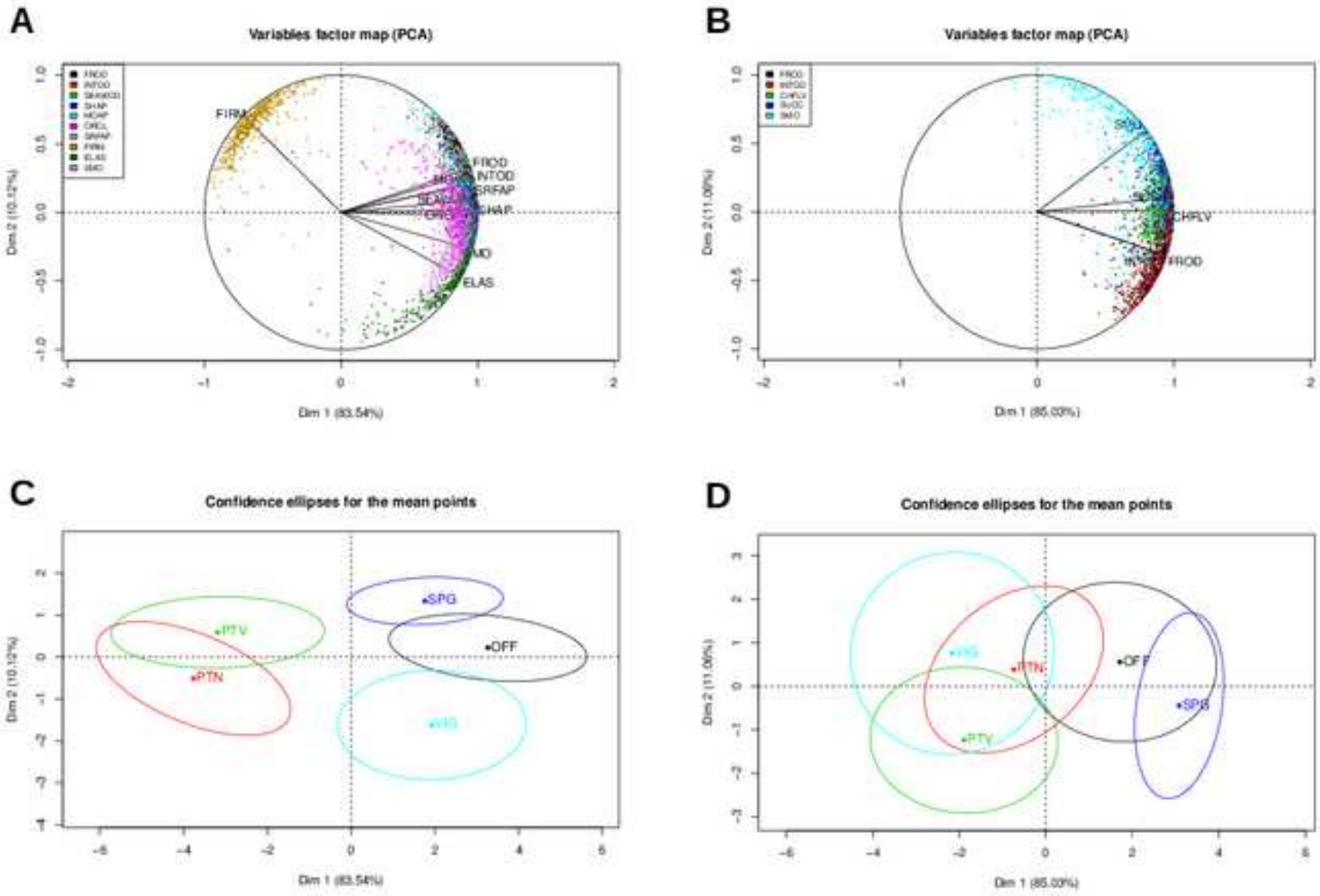
Average and standard-deviation values are expressed in relative percentage of total lipids (equivalent to g $100g^{-1}$ DW). Samples of PTN and OFF for lipid classes n=3; for VIG n=2. Totals include some minor components not shown.

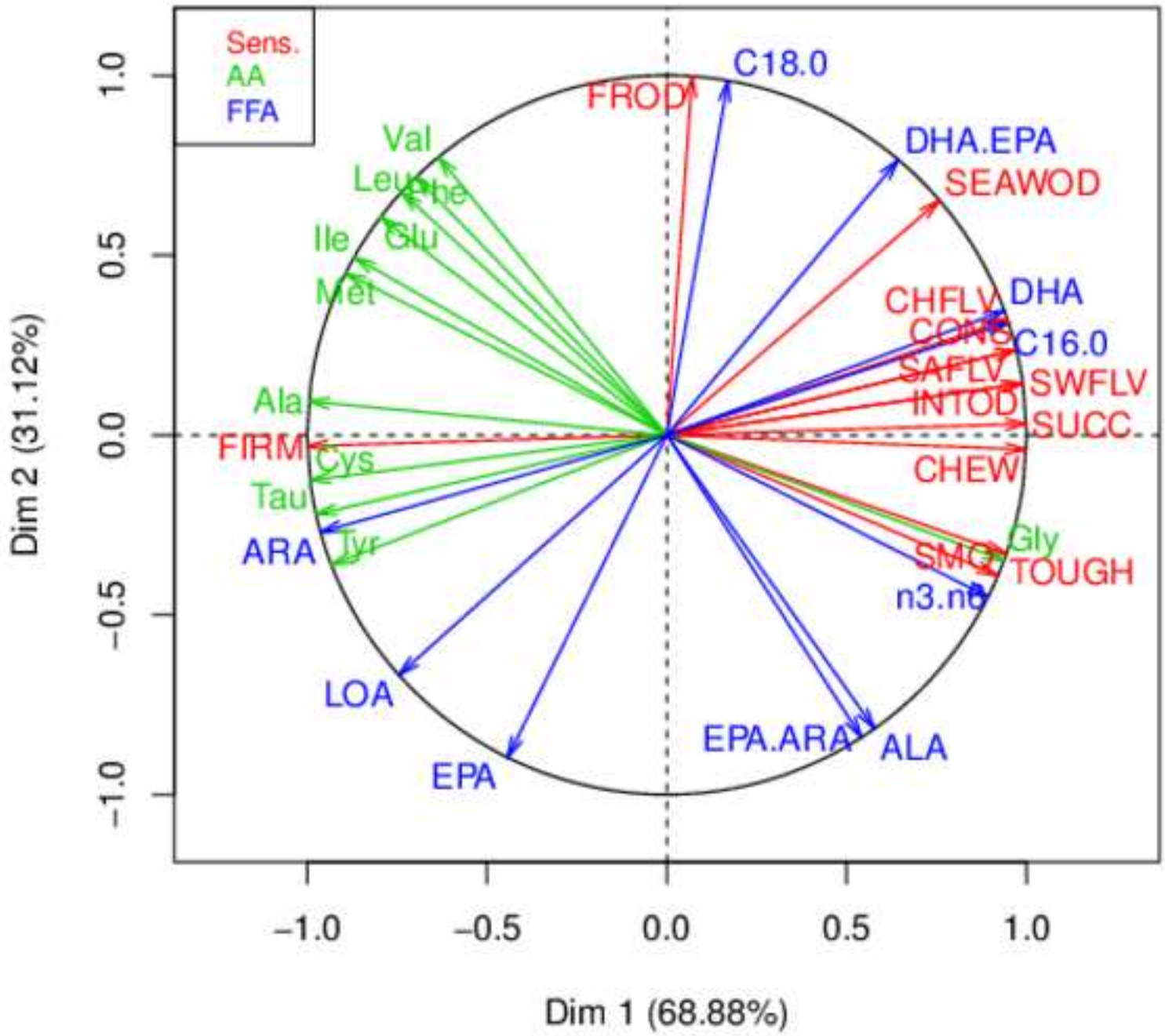
Different letters indicate significant differences for $p<0.05$ (LSD post-hoc test; * Games-Howell post-hoc test). ALA – alpha-linolenic acid; ARA – arachidonic acid; DHA – docosahexaenoic acid; DMA – dimethyl acetal derivates; EPA – eicosapentaenoic acid; LA – linoleic acid; MUFA – monounsaturated fatty acids; *n*3/*n*6– omega-3/omega-6 fatty acids ratio; PUFA – polyunsaturated fatty acids; SFA – saturated fatty acids; UK – unidentified/unknown.

Table IV - Free amino acids profiles of North Portugal (PTN), Offshore (OFF) and Vigo (VIG) mussels.

	Free amino acids (μmol g^{-1} DW)	PTN	OFF	VIG
Essential	Histidine (His)*	30.16 ± 2.35^{b}	17.89 ± 0.81^{a}	52.23 ± 8.99^{b}
	Isoleucine (Ile)	36.99 ± 1.29^{c}	11.09 ± 0.81^{a}	32.07 ± 1.53^{b}
	Leucine (Leu)	39.40 ± 0.87^{c}	11.39 ± 0.86^{a}	26.82 ± 1.93^{b}
	Lysine (Lys)	67.67 ± 6.69^{a}	73.44 ± 5.74^{a}	120.36 ± 6.39^{b}
	Methionine (Met)	35.59 ± 3.71^{b}	15.04 ± 0.94^{a}	32.73 ± 2.37^{b}
	Valine (Val)	64.04 ± 2.77^{c}	22.03 ± 1.30^{a}	42.17 ± 0.80^{b}
	Threonine (Thr)	52.20 ± 5.07^{b}	26.94 ± 2.64^{a}	78.80 ± 11.00^{c}
	Phenylalanine (Phe)	11.54 ± 0.65^{c}	5.71 ± 0.22^{a}	9.22 ± 0.34^{b}
	Tryptophan (Trp)	14.48 ± 1.30^{b}	7.69 ± 1.15^{a}	15.43 ± 0.47^{b}
Non-Essential	Arginine (Arg)	113.57 ± 7.79^{a}	160.51 ± 4.08^{b}	200.67 ± 4.72^{c}
	Glycine (Gly)*	780.59 ± 18.58^{a}	1648.65 ± 80.55^{b}	801.49 ± 22.98^{a}
	Tyrosine (Tyr)	35.33 ± 2.88^{b}	19.05 ± 0.96^{a}	67.10 ± 1.66^{c}
	Proline (Pro)	53.73 ± 2.62^{b}	46.76 ± 2.41^{a}	57.97 ± 1.80^{b}
	Glutamine (Gln)	53.61 ± 0.16^{a}	56.13 ± 2.98^{a}	166.16 ± 8.26^{b}
	Alanine (Ala)*	350.78 ± 14.97^{b}	189.13 ± 2.95^{a}	404.75 ± 26.78^{b}
	Asparagine (Asn)*	12.78 ± 0.18^{a}	27.46 ± 1.85^{b}	78.14 ± 2.46^{c}
	Aspartic Acid (Asp)	17.96 ± 4.72^{a}	31.42 ± 22.11^{ab}	57.25 ± 14.32^{b}
	Glutamic Acid (Glu)	224.38 ± 14.16^{b}	166.74 ± 9.67^{a}	205.94 ± 8.28^{b}
	Serine (Ser)	78.26 ± 3.28^{a}	80.58 ± 6.24^{a}	172.11 ± 12.61^{b}
	Alpha-amino-butyric-acid- (α-ABA)	14.16 ± 1.22^{a}	16.86 ± 0.90^{b}	16.62 ± 0.99^{b}
	Beta-Alanine (β-Ala)	17.40 ± 1.03^{a}	25.40 ± 0.33^{b}	16.40 ± 2.03^{a}
	Phosphoserine (Pser)	12.44 ± 0.35^{c}	9.72 ± 0.37^{b}	8.42 ± 0.23^{a}
	Hydroxy-proline (HyPro)	17.63 ± 1.97^{b}	5.35 ± 0.13^{a}	6.25 ± 1.41^{a}
	Ornithine (Orn)	17.06 ± 1.24^{b}	7.84 ± 0.50^{a}	25.58 ± 1.00^{c}
	Taurine (Tau)	1818.93 ± 46.95^{a}	1702.03 ± 88.72^{a}	1950.68 ± 53.58^{b}
	Total	3988.53 ± 38.29^{a}	4407.79 ± 159.80^{b}	4665.20 ± 170.08^{c}

Values are expressed in μmol g^{-1} DW. Samples n=3.
Different letters indicate significant differences for $p<0.05$ (LSD *post-hoc* test; * Games-Howell *post-hoc* test).




Sens.
AA
FFA
C18.0
FROD
DHA.EPA
SEAWOD
Val
Leu
Phe
Ile
Glu
Met
CHFLV
DHA
COOK
C16.0
SAFLV
SWFLV
INTOD
SUCC.
Ala
FIRM
Cys
CHEW
Tau
ARA
Tyr
Gly
SMOO
TOUGH
n3.n6
LOA
EPA.ARA
ALA
EPA
Dim 2 (31.12%)
Dim 1 (68.88%)
1.0
0.5
0.0
−0.5
−1.0
−1.0
−0.5
0.0
0.5
1.0

Highlights:

- Offshore Portugal mussel culture compared to NW Iberia inshore sites of production
- The production sites influenced the size and nutritional content of mussels
- A sensory analysis panel was able to distinguish mussels of different origins to some extent
- Mussels produced off the Algarve coast should have good acceptability by consumers