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ORIGINAL RESEARCH

A sensory and nutritional validation of open ocean mussels (*Mytilus galloprovincialis* Lmk.) cultured in SE Bay of Biscay (Basque Country) compared to their commercial counterparts from Galician Rías (Spain)

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Abstract Mussel shell biometry, nutritional quality as well as consumer sensory evaluation of experimental open ocean cultured mussel *Mytilus galloprovincialis* (Lamarck 1819) were analyzed and compared to that of commercial mussels from Galician Rías available in the local market. Both mussel products were of the same commercial size. In this study, open ocean mussels were significantly higher and wider than those of Galician Rías. In addition, with the exception of ash content, both mussel products showed similar biochemical composition. Regarding fatty acid profiles, however, statistical differences were detected. These differences were not fully reflected in the sensory assessment. In terms of consumer acceptability, both mussel products were considered equally satisfactory.

Keywords Fatty acids · Mussel · *Mytilus galloprovincialis* · Open ocean aquaculture · Proximate composition · Sensory analysis

Introduction

Aquaculture is a seafood supply that has increased gradually over the last few decades (Langan and Horton 2003). This growth is due to progress in technology, changes in policy, and an increased societal awareness of sustainability (Bushek et al. 2004; Coen et al. 2007; Costa-Pierce 2008; Forrest et al. 2009; Bostock et al. 2010). In particular, mollusk aquaculture represents more than 75% (13.9 million tons) of the world's aquaculture, with mussel production representing around 13% (1.8 million tons) of its annual production (FAO 2014). Spain, France, and the Netherlands produce one-third of the global mussel aquaculture production (Buck et al. 2010). Specifically, Spain, with an average production near 200,000 tons per year, is the second largest producer worldwide and the first in Europe (FAO 2014). A large part (95%) of its annual production is produced in the Galician Rías from the SW Bay of Biscay (Galician region). Since 2008, mussel products from that area have had a Protected Designation of Origin certifying quality and traceability within the standards of the EU seafood policy.

Shellfish species are a valuable food source in the socioeconomic context of the Basque Country (SE Bay of Biscay) (Gracia 1996), yet mussel aquaculture is limited. Indeed, over the last few decades, every

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mollusk (especially bivalves) consumed in this area has been imported. The lack of development of an aquaculture or shellfish sector in the region of the Bay of Biscay is mainly linked to high wave-energetic scenarios (Galparsoro et al. 2012) and previous issues related to estuarine water quality (Chust et al. 2009). A further restriction is current legislation, which aims to prevent conflict between users of marine habitats. These conflict-prevention methods hinder the development of near-shore aquaculture as the limited inshore spaces are protected marine habitats (Galparsoro et al. 2010; Borja et al. 2011; Pascual et al. 2011).

In addition to these local limitations, the main obstacles in mussel aquaculture in general are those regarding infectious diseases and parasites (Bower et al. 1994), marine phycotoxins (Svensson 2003; Moroño et al. 2003) as well as carrying capacity and competition for the use of marine space (Martínez et al. 2007; Costa-Pierce 2008; Byron et al. 2011). Limited marine space coupled with commercial objectives often results in non-sustainable practices for commercial mussel cultivation (Smaal 2002; Waite et al. 2005; Duarte et al. 2008). It is encouraging, however, that ecosystem-based approach-management strategies have been recommended to promote the sustainable development of mussel cultivation (Soto et al. 2008).

One such strategy is open ocean aquaculture which represents a promising alternative for the sustainable cultivation of bivalve species (Polk 1996; Hesley 1997; Stickney 1998, Bridger and Costa-Pierce 2003). However, this activity is still in development stages (Hickman 1992), and main mussel culture techniques (i.e., rafts, pole racks and/or longline systems) have been established almost exclusively in protected near-shore waters (Pérez-Camacho et al. 1991; Myrand et al. 2009) or in estuarine habitats (Penney et al. 2002; LeBlanc et al. 2005; Drapeau et al. 2006). Therefore, few studies have reported successful biological and/or engineering results on open ocean mussel aquaculture to date (Langan 2001; Langan and Horton 2003; Buck 2007).

Open ocean aquaculture is not only a financially attractive but also an environmentally sound one with a highly nutritious end product for the consumer. Indeed, there is an increasing demand for products sourced from open ocean aquaculture methods, resulting in increased revenue in international markets (Orban et al. 2002; Fuentes et al. 2009; Pogoda et al. 2013). Further, developing open ocean aquaculture methods will be environmentally beneficial (Shumway et al. 2003), as mussels and other bivalves are filter feeders, capturing and eating microscopic particles, plants, and nutrients by filtering, and thus cleaning ocean water (Shpigel et al. 1993). For the consumer, as most farmed mussels have fast growth, they provide year-round nutritious food, much in demand (Seed and Suchanek 1992; Gosling 2003). Mussels are appreciated by humans for their organoleptic properties (Grienke et al. 2014) as well as for their health benefits. They provide high-quality protein, minerals, essential trace elements, fat-soluble vitamins (i.e., vitamin D), and essential fatty acids EFA, especially long-chained polyunsaturated fatty acids: omega-3 (ω3 LC-PUFAs) (Stankovic et al. 2011; James 2013).

Regarding consumption, mussel shape and appearance are decisive factors for consumers' purchase (Fuentes et al. 2009), whereas meat quality (and hence, apparent health benefits) is regulated by biochemical composition (Orban et al. 2002; Filgueira et al. 2006; Fuentes et al. 2009). Mussel meat quality has been described by consumers as the result of not only chemical and biological characteristics, but also organoleptic properties, such as the appearance of the muscle, the intrinsic flavour, and the absence of undesirable components (Vernocchi et al. 2007). As reported by Fuentes et al. (2009) mussel organoleptic characteristics can be origin dependent. This means that different production sites, with different conditions and culture technologies, are expected to promote changes in growth performance and biochemical composition of mussel individuals, which could in turn reflect different product qualities at consumer level (Oliveira et al. 2015).

To date, no studies have investigated organoleptic properties on open ocean mussel products from the SE Bay of Biscay. Both biochemical composition and organoleptic properties are of special importance to anticipate consumer acceptance and market feasibility (Gökoglu 2002; Oliveira et al. 2015) before considering any implementation of mussel activity in the region.

The specific goals of this study were to characterize and compare the biometric parameters, nutritional content, and sensory aspects between experimental mussel product from the SE Bay of Biscay (open ocean) and commercial mussel product from the SW Bay of Biscay (Galician Rías).

Materials and methods

Location and sampling

An experimental suspension longline culture system (Langan 2000) was developed to cultivate shellfish in the open waters of the south eastern corner of the Bay of Biscay (Basque region; Spain). This region extends over 150 km and it is oriented E–W. The climate is temperate, oceanic, with moderate winters and warm summers. According to Köppen's classification, the area is associated with a marine temperate climate (Cfb) (Collins and Borja 2004). Seeds (17.40 ± 5.07 mm) were naturally collected during June 2013 and cultivated at the experimental site (43°21.39′N; 02°26.90′W) over a 1-year period. The open ocean site is already classified as a Class A production area (according to EU regulation (EC) 854/2004). Therefore, those mussels are not subjected to depuration requirements. The site is located at 2 nautical miles off the coast without any protection from land masses (Ryan 2004). Galparsoro et al. (2012) described strong hydrodynamic conditions on Basque Country's coast. A detailed description of current and waves in the experimental site can be found in Azpeitia et al. (2016).

Mussel individuals were cultured within 17 m water column depth, on mussel-growing ropes of 12 m in length. Mussel ropes hung from 5 m headline depth, with a distance between ropes of 0.5 m and an initial stocking density of 681 ± 37 individuals per lineal meter. After 13 months of cultivation, in July 2014, 200 mussel specimens of "commercial size" (Peteiro et al. 2006, i.e., 70 mm) were carefully detached from the ropes by cutting the byssal threads and cleaned of encrusting organisms. Selected mussel individuals were transported in plastic self-draining boxes covered by flaked ice. Later in the laboratory, mussels were conserved in plastic boxes, stored at 4 °C and covered with flaked ice for 24 h previous to analysis to preserve them. Similarly, 200 mussel individuals from Ría de Arosa (Galician Region; Spain) with a comparative commercial size (69.65 ± 6.11 mm) were obtained at early hours on the morning of the day of the sensory analysis. Commercial mussels were bought from a local seafood retailer to ensure a comparable size product with the same conservation procedure (plastic box and stored at 4 °C). These mussels were also stored at 4 °C and covered with flaked ice for some hours previous to analysis. Subsequently, two stocks of 50 mussel individuals from Galicia and from the experimental open ocean site were frozen at −80 °C to await morphometric and biochemical analysis.

Morphometry

Twenty mussel individuals from each origin (experimental open ocean and commercial) were carefully detached by cutting the byssal threads, cleaned of encrusting organisms and measured for morphometric analysis. The individuals were measured for their shell length (L; maximum anterior–posterior axis), shell height (H; maximum dorso-ventral axis), and shell width (W; maximum lateral axis) using a digital caliper to the nearest 0.1 mm. The biometric terminology used in this study is that described by Seed (1968). Mussel shape was analyzed by linear allometric relation between log-transformed mussel shell dimensions [length (L), height (H) and width (W)].

Biochemical composition and fatty acids

Three replicate samples of 10 pooled mussel individuals from each origin were considered for biochemical characterization (including proximate and glycogen analysis) and lipids determination (including total lipids and fatty acids), respectively.

The fresh individuals were first minced in a food processor (IKA® M 20 universal mill, IKA 1603601, Germany). Moisture and ash were determined according to the gravimetric determination. Briefly, minced tissue pools were placed in pre-weighed porcelain trays for drying at 80 °C for 24 h and then weighed to the nearest 0.001 g. Subsequently, dry mussels' tissue pools were ashed at 450 °C for 4 h in a muffle furnace, cooled in a desiccator and weighed again to determine the ash-free dry weight content.

Total protein was determined according to the Kjeldahl's method (AOAC 1975). Samples were digested in a digestor BUCHI B-435, distilled using a Rotavapor® R-210 (BÜCHI Labortechnik AG, Switzerland) and automatic volumetric titration developed through a Mettler Toledo T- 50 (Mettler Toledo, United States).

Glycogen concentration ($mg \cdot g^{-1}$ mussels' tissue) was calculated based on a mussel glycogen standard (Sigma, Saint Louis, MO) following the methodology reported by Gallardi et al. (2014). Briefly, 30% KOH was added to 0.5 g of homogenized mussels' tissue (ratio 2:1). The samples were then heated in a shaking-water bath at 100 °C for 20 min, vortexed for 30 s and subsequently chilled on ice for 5 min. After cooling, 200 µL of each sample was transferred to a 4 mL glass vial followed by 200 µL of 95% ethanol. The solution was vortexed again briefly and then placed in a boiling-water bath for 15 min followed by the addition of 1.2 mL of lukewarm water. The samples were again briefly vortexed and then allowed to stand at room temperature for 5 min before measuring the glycogen content. Glycogen content was measured by colorimetric reaction using 25 µL of prepared sample. 10 µL of 80% aqueous phenol and 200 µL of sulphuric acid were added to the samples on 96-well plates. Absorbance was measured using a multi- detection microplate reader (Synergy HT, BIO-TEK) at 490 nm. The concentration of glycogen in the samples was calculated based on a mussel glycogen standard (Sigma, Saint Louis, MO).

Total lipids were determined based on Blight and Dyer method (Hanson and Olley 1963). In this way, total lipids from a known weight of tissue was extracted and purified by homogenization in a suitable excess of chloroform/methanol/water (2:2:1.8 v/v/v). Following this, total lipids in chloroform solution were determined by weighting the eluate after removal of the solvent by rotary evaporation. All lipid extracts were then stored in chloroform at −30 °C to await analysis.

Fatty acid methyl esters (FAME) were separated and quantified using an Agilent 7890 Gas Chromatographer equipped with auto-injector (Agilent Technologies, United States). The gas chromatograph is equipped with a flame-ionization detector (290 °C) and a DB-WAS 122-7032 Agilent Technologies capillary column (30 m _ 0.25 mm I.D., film 0.25 µm). Helium was used as a carrier gas and initial oven temperature was 50 °C, followed by an increase at a rate of 10 $°C \cdot min^{-1}$ to a final temperature of 240 °C for 9 min. Individual FAME were identified by comparison of retention times with four commercial fatty acid standards (PUFA 1, PUFA 3, BAME and 37-component) supplied by Supelco (Bellefonte, PA) for area percent normalization. At each sample relative quantities were expressed as weight percent of total fatty acids.

Sensory evaluation/consumer testing

Thirty regular domestic consumers of mussel were randomly recruited by email from AZTI's database. They were segmented by gender (50% men) and age range (18–70 years). To remove debris and any remaining byssal thread, 150 mussels were washed and scrubbed for the sensory analysis. Samples were steamed at 100 ± 1 °C for 1 min and then served at 65 ± 1 °C. Mussels were presented to consumers on white plastic plates labeled with 3-digit random numbers, containing two mussels from each origin (experimental from open ocean and commercial from Galician Rías). Consumers tasted one mussel from each origin. The consumers received oral and written instructions and were provided with water and unsalted-crisp bread for palate cleansing after each tasting. Each consumer tasted the samples in randomized order and evaluated five attributes (appearance, odor, taste, texture, and global acceptance) with a structured 9-point scale ranging from 1 (equivalent to I dislike it very much) to 9 (equivalent to I like it very much). The 9-point hedonic scale (Peryam and Girardot 1952; Peryam and Pilgrim 1957) is the most commonly used scale for testing consumer preference and acceptability of foods (Schutz and Cardelo 2001). This kind of scales produce central tendency effects on panelist, which reduces the options to a 7-point scale (Villanueva et al. 2005). Despite this possible reduction, a 9-point scale with an arithmetic adjustment can always be straightforward re-scaled to a 7-point scale (Epler et al. 1998). This enables the comparisons with different scale formats with less response options (Dawes 2012), which are common in current scientific literature. Consumers' purchase intention was also assessed with a structured 5-point scale ranging from 1 (equivalent to I would not buy it) to 5 (equivalent to I will buy it). The same test was repeated three times with each panelist. Consumers tasting was performed in the sensory laboratory of AZTI- Tecnalia specifically designed according to ISO 8587:2006.

Statistical analysis

Relative shell morphology was investigated through the general linear function, where y and x are size-related measures and *a* and *b* are constants (i.e., intercept and slope; which represents the coefficient of relative shape)

(Pauly 1983). Similarly, allometric equations were generated separately for mussels from each origin, using linear regression and logarithmic transformation. The differences between allometric coefficients (b) from each origin were evaluated using ANCOVA (Rohlf and Sokal 1981).

Means and standard deviations (mean ± SD) of data on morphometrics, proximate composition (humidity, ash, protein, glycogen, and lipid content), as well as fatty acid profiles, of mussels were plotted using Sigmaplot (11.0) statistical and graphical software (Systat software). Statistical analyses were carried out using a statistical package (SPSS 20.0 for Windows®). Sensory attributes and evaluation data were analyzed using the R-project software (R Development Core Team 2008). A significance level of 0.05 was used. Normality was tested by Shapiro–Wilk test and the homoscedasticity was checked using Leven's test. The differences between mussel products type (experimental and commercial products) were compared using independent sample t test. Arcsine transformation (Zar 1984) was applied to the data expressed as percentage.

Results

Morphometry

The biometric results of the two mussel products (experimental open ocean and commercial from Galician Rías) tested within the present study are shown in Table 1. No statistical differences between the shell lengths (L) of both mussel products were detected. Conversely, significant differences were detected for the measurements of shell height (H) and shell width (W) between both mussel products. As observed, the open ocean mussel individuals from the present study displayed significantly higher morphometric values than those from the commercial samples.

The allometric relationships between shell length (mm) and shell width (mm), shell length and shell height (mm), or between shell width (mm) and shell height (mm) of both mussel products are shown in Table 2. Based on ANCOVA analysis (Table 3), the factor of product type significantly affected both the linear relations between log-transformed shell length (mm) and log-transformed shell height (mm), as well as the linear relation between log-transformed shell length (mm) and log-transformed shell width (mm). Higher values were found in the experimental mussels. Finally, no ANCOVA analysis was performed to compare the linear relation between log-transformed shell height (mm) and log-transformed shell width (mm) as significant interaction between the factor (product type) and the covariable [log-transformed shell width (mm)] was observed. Hence, no direct relation between the change in shell width (mm) and the change in shell height (mm) among mussel products could be assessed.

Table 1 *M. galloprovincialis*. Biometric characteristic of the mussel shell and proximate composition of mussel tissue pools from experimental open ocean (SE the Bay of Biscay; Basque region) and sheltered Rías (SW Bay of Biscay; Galician region) conditions

	Experimental	Commercial	One-way ANOVA	
	Open ocean	Galician Rías	F	P value
L (mm)	70.29 ± 7.96	69.65 ± 6.11	0.037	0.849
H (mm)	37.56 ± 4.59*	33.58 ± 2.48	10.471	0.003
W (mm)	24.58 ± 4.04*	21.18 ± 4.59	5.538	0.024
Moisture (%)	78.77 ± 1.54	76.57 ± 1.77	2.661	0.178
Protein (dry weight %)	49.52 ± 4.29	44.79 ± 4.02	1.962	0.234
Lipid (dry weight %)	6.27 ± 1.47	6.30 ± 1.34	0.001	0.976
Ash (dry weight %)	13.00 ± 1.16*	9.40 ± 0.82	19.253	0.012
Carbohydrates (dry weight %)	31.21 ± 4.87	39.51 ± 6.07	3.356	0.141

Mean ± standard deviation, $n = 20$

* Statistical significance was set up at ($P < 0.05$) (one-way ANOVA)

Table 2 *Mytilus galloprovincialis*. Parameter of shell shape (length (L), width (W) and height (H)) regression equations used to relate shell length (mm) to shell height (mm) or shell width (mm), and shell width (mm) to shell height (mm) of different mussel products (experimental vs. commercial); equation fitted to $\log_{10} X = \log_{10} a + b \log_{10} Y$, where a intercept and b slope, and X and Y shell measurements. Error of correlation coefficient (r), number of specimens (n) used to derive equation are also given

Allometric relation	Mussel product		*B*	SE	*t*	Sig	95% CI		R^2	*N*
							Upper	Lower		
Log H/Log L	Experimental	Intercept	–0.185	0.224	–0.823	0.421	–	0.287	0.773	20
		Slope	0.952	0.122	7.834	0.000	0.697	1.208		
	Commercial	Intercept	0.390	0.241	1.620	0.123	–0.116	0.897	0.552	20
		Slope	0.616	0.131	4.709	0.000	0.341	0.891		
Log W/Log L	Experimental	Intercept	–0.874	0.284	–3.075	0.007	–	–	0.882	20
		Slope	1.225	0.154	7.951	0.000	0.901	1.549		
	Commercial	Intercept	–0.501	0.243	–2.066	0.053	–1.011	0.008	0.873	20
		Slope	1.000	0.132	7.595	0.000	0.724	1.277		
Log H/Log W	Experimental	Intercept	0.735	0.161	4.557	0.000	0.396	1.073	0.775	20
		Slope	0.604	0.116	5.197	0.000	0.360	0.848		
	Commercial	Intercept	0.790	0.149	5.287	0.000	0.476	1.104	0.757	20
		Slope	0.548	0.111	4.917	0.000	0.314	0.782		

Table 3 *Mytilus galloprovincialis*. Mussel product type effects on shell length (mm) to shell width (mm) and on shell length (mm) to shell height (mm), allometric relations analyses. Regression equation exponent b (slope) were compared using ANCOVA (Rohlf and Sokal 1981), with α set at 0.05

Allometric relation	Source of variation	ANCOVA			R^2
		df	*F*	Sig	
Log H/Log L	Log length (covariable)	1	83.561	0.000	0.759
	Product type (factor)	1	29.992	0.000	
	Error	37			
Log W/Log L	Log length (covariable)	1	121.922	0.000	0.803
	Product type (factor)	1	19.953	0.000	
	Error	37			

Biochemical composition and fatty acids

The proximate composition of both mussel products is shown in Table 1. Moisture levels were relatively high within both samples. Mussels from commercial origin (Galician Rías) showed slightly higher moisture values (78.77% vs. 76.57%) than mussels from the open ocean conditions of the present study, yet significant differences were not detected.

Significantly lower ash levels were detected within the commercial individuals (13.00% vs. 9.40%) compared to the study ones.

Lipid contents were slightly higher in the commercial samples (6.27% vs. 6.30%), although no significant differences were found. Protein contents did not show any significant differences between mussel products. Finally, glycogen levels (71.06 ± 16.71 mg·g^{-1} and 100.59 ± 25.05 mg·g^{-1} at experimental and commercial mussels, respectively) were slightly higher in commercial samples, although differences were not statistically significant.

The FA composition of both mussel products are shown in Table 4. The saturated fatty acids (SAFA) accounted for 29% in experimental mussels and significantly lower content (25%) for commercial mussels. Saturated fatty acid predominated over the monounsaturated fatty acid (MUFA) with 12% for the experimental and significantly higher level (22%) for the commercial samples. Both mussel products showed the

Table 4 *M. galloprovincialis*. Fatty acid compositions (percentage of total fatty acids) of mussel tissue pools analyzed during the comparative study between the experimental open ocean mussels from the SE Bay of Biscay and commercial mussels obtained from the sheltered Rías from Galicia

Fatty acids	Experimental open ocean	Commercial Galician Rías
14:0	2.52 ± 0.80*	4.14 ± 0.60
15:0	0.57 ± 0.07*	0.36 ± 0.02
16:0	18.91 ± 1.29*	15.50 ± 0.43
17:0	1.90 ± 0.32*	1.12 ± 0.20
18:0	4.70 ± 0.93	3.74 ± 0.52
20:0	0.06 ± 0.05	0.02 ± 0.04
22:0	0.03 ± 0.03	0.18 ± 0.03*
∑SAFA	28.69*	25.05
14:1	0.42 ± 0.07	0.99 ± 0.17*
16:1ω7	2.40 ± 0.87	11.14 ± 1.42*
16:1ω5	0.30 ± 0.05	0.25 ± 0.02
17:1	0.19 ± 0.16	0.40 ± 0.10
18:1ω9	2.22 ± 0.68	1.14 ± 0.05
18:1ω7	1.22 ± 0.15	3.19 ± 0.07*
20:1 ω11	1.21 ± 0.23	1.32 ± 0.17
20:1ω9	3.48 ± 0.28*	1.59 ± 0.19
20:1ω7	0.47 ± 0.05	2.03 ± 0.16*
22:1	0.06 ± 0.06	0.24 ± 0.14
∑MUFA	11.98	22.28*
16:4ω3	8.32 ± 2.25	3.04 ± 4.70
18:2ω6	2.03 ± 0.32*	0.87 ± 0.02
18:3ω3	1.82 ± 0.44*	0.77 ± 0.09
18:4ω3	3.74 ± 1.23*	1.35 ± 0.24
20:2 α	2.76 ± 0.36*	1.57 ± 0.31
20:2 β	0.25 ± 0.04	1.14 ± 0.35*
20:2ω6	0.71 ± 0.12*	0.38 ± 0.03
20:4ω6	1.30 ± 0.21	2.61 ± 0.12*
20:4ω3	0.34 ± 0.13	0.64 ± 0.23
20:5ω3	9.85 ± 1.06	24.71 ± 2.21*
22:2 NMIDa	0.73 ± 0.10*	0.12 ± 0.10
22:2 NMIDb	1.22 ± 0.13	4.54 ± 0.43*
21:5ω3	1.20 ± 0.16	1.09 ± 0.06
22:4ω6	0.20 ± 0.04	1.90 ± 1.54
22:5ω6	0.61 ± 0.03*	0.15 ± 0.15
22:5ω3	1.12 ± 0.11	2.13 ± 0.21*
22:6ω3	23.13 ± 2.20*	5.66 ± 0.47
∑PUFA	59.33*	52.67
NMID	1.95	4.66*
Terrestrial (18:2ω6 + 18:3 ω3)	3.85*	1.64
Ω3/Ω6	10.21	6.67
DHA/EPA	2.35*	0.23

Values (mean ± SD, $n = 10$)

NMID Non-methylene-interrupted dienoic fatty acid

* Statistical significance was set up at ($P < 0.05$) (one-way ANOVA)

majority of fatty acids consisted of polyunsaturated fatty acids (PUFA), with significantly higher level (59%) for experimental mussels than for commercial mussels (52%).

In the case of SAFA, palmitic acid (16:0) was predominant (18.91 and 15.50%) in both products, followed by stearic acid 18:0 in the open ocean mussels (4.70%), and by myristic acid 14:0 in the commercial mussels (4.14%). Significant differences (one-way ANOVA; $P < 0.05$) in the SAFA profile (i.e., the sum of pentadecanoic 15:0, palmitic 16:0 and behenic 22:0 acids) were found between both mussel products.

Regarding MUFA, the commercial mussel from Rías displayed abundant related palmitoleic acid 16:1ω7 (11.4%). This was followed by vaccenic acid 18:1ω7 (3.19%) and eicosenoic acid 20:1ω7 (2.03%). Conversely, the observed trend of MUFAs in the open ocean experimental individuals was not equivalent; the major acid was gondoic acid 20:1ω9 with 3.48%, followed by palmitoleic acid 16:1ω7 with 2.40% and oleic acid 18:1ω9 with 2.20%. Thus, significant differences (one-way ANOVA; $P < 0.05$) were found between MUFA of both mussel products.

Regarding PUFAs, eicosapentaenoic (EPA) (20:5ω3) and docosahexaenoic (DHA) (22:6ω3) acids were the most important. Commercial mussels showed higher EPA (20:5ω3) with 24.71% and less DHA (22:6ω3) with 5.66%, whereas the experimental open ocean mussels showed less EPA (20:5ω3) content with 9.85% and higher (DHA (22:6ω3) with 23.13%. Thus, the DHA/EPA ratios were significantly different (one-way ANOVA; $P < 0.05$) between mussel products.

Non-methylene-interrupted dienoic fatty acid (i.e., 22:2 NMIDa and 22:2 NMIDb) were higher in the commercial samples (4.66%) than in the experimental ones (1.95%). Differences at NMID level were statistically significant (one-way ANOVA; $P < 0.05$) between mussel products.

Finally, terrestrial fatty acids (i.e., 18:2ω6 + 18:3 ω3) accounted for the 3.85% in experimental mussels and 1.64% in the commercial ones; differences between mussel products were statistically significant (one-way ANOVA; $P < 0.05$).

Sensory evaluation/consumer testing

The panelist male:female ratio (%) participating in the present study was 37.7:63.3. The range of ages between participants was distributed as follows: 18–30 years (13.3%); 31–40 years (40%); 41–50 years (33%); 51–60 years (13.3%). The 77% of participants were regular mussel consumers (3.3% more than once a week; 26.7% once a week; 46.7% once a month), whereas the 23.3% declared to be occasional mussel consumers.

The resulted acceptance for both mussel products was around 7 points out of 9 (Fig. 1). No significant differences (t test; $P > 0.05$) were detected for any of the sensory attributes analyzed between both products (Table 5). The results of the qualitative assessment showed that both mussel products showed good appearance and odor. The results of the qualitative assessment showed that both mussel products showed good appearance and odor. Particularly, the experimental ones showed to provide more intense sea-flavour with a slightly softer and more juicy texture. Some other aspects such as the whitish color on the muscle tissue and/or the salty taste were criticized when observed. A large proportion of the panelists (70%) expressed potential interest in purchasing the experimental product, while a small minority (17%) did not. The remaining 13% of panelists did not express a purchase preference. For the commercial mussels, 83% of the panelists showed purchase intention, while 10% showed uncertainty (or doubt) and 7% would not buy it (Fig. 2).

Discussion

All mussels consumed in the Basque region, during recent decades, are imported (Gracia 1996). Currently, the only way to cultivate mussels in the Basque Country is offshore and with submerged longline method (Azpeitia et al. 2016). The aim of the study was to compare if experimental open ocean cultured mussels of commercial size were as competitive as local market leader of the same commercial size. No significant differences were observed in shell length between both products, which validated the analysis of quality differences among mussel products as well as the analysis of consumer perception among products. Although panelists were not able to inspect mussel shells, only mussels without visible damage (open valves or broken shell) were used in the study. Direct influences on the appearance of the product should be derived from the differences found at biometric levels between both mussel (*M. galloprovincialis* Lmk.) origins. Different shell

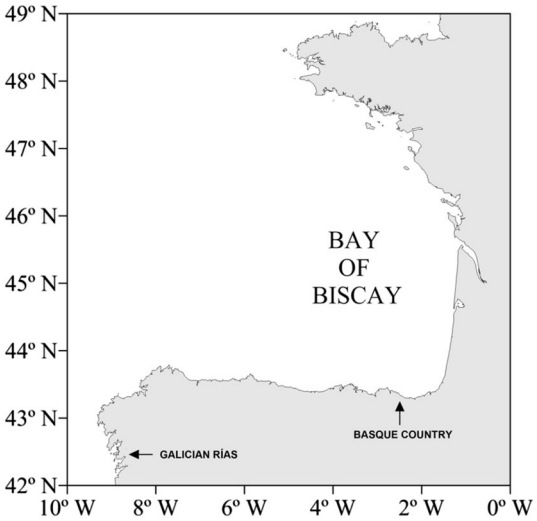

Fig. 1 Map of the Bay of Biscay showing the specific location of the open ocean experimental site at the SE Bay of Biscay (Basque Country) and the commercial mussel product from the SW Bay of Biscay (Galician Region)

Table 5 *M. galloprovincialis*. Student's *t* analysis carried out for all sensorial attributes analyzed in experimental mussel from the open ocean conditions of the SE Bay of Biscay and mussels cultured in at sheltered Rías from Galicia

	Experimental open ocean	Commercial Galician Rías	Student's *t*	
			t	*P* value
Appearance	6.467 ± 2.097	7.033 ± 1.066	1.32	0.194
Odor	6.967 ± 1.402	7.3 ± 1.088	1.029	0.308
Flavor	7.533 ± 1.106	7.4 ± 1.303	−0.427	0.671
Texture	7.033 ± 1.586	7.533 ± 0.973	1.472	0.148
Global impression	6.9 ± 1.517	7.333 ± 1.269	1.2	0.235

Values (mean ± SD, $n = 30$)

* Statistical significance was set up at ($P < 0.05$) (pairwise comparison)

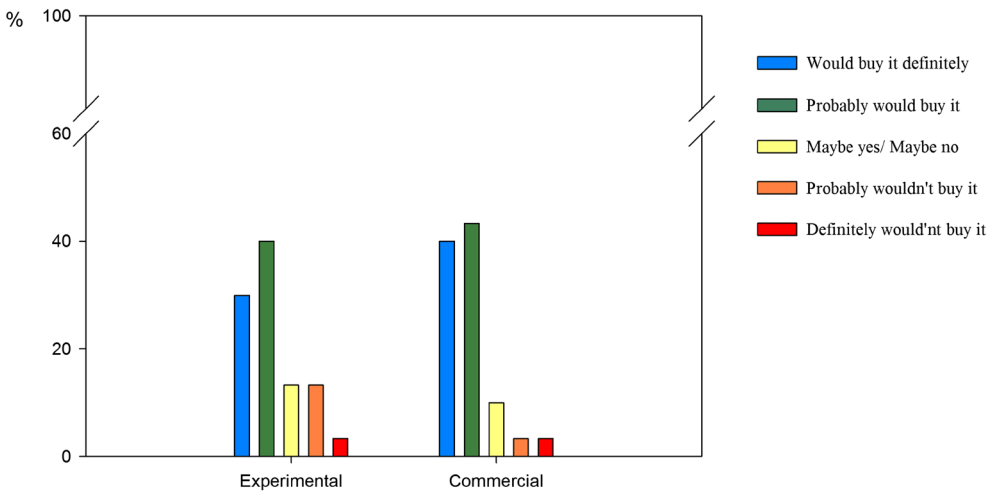

Fig. 2 *M. galloprovincialis*. Buying-intention results carried out on the comparison between mussels from the open ocean in the SE Bay of Biscay ("experimental") and from sheltered estuaries in the SW Bay of Biscay ("commercial")

shapes among mussel product were observed. Mussels cultured in Galician Rías were found to be thinner (reduced W. L^{-1} ratio) and lower (reduced H. L^{-1} ratio) than experimental open ocean mussels. Commercial mussels may have been cultured at higher densities than experimental mussels. Stocking density on experimental mussels was 681 individuals/m, whereas commercial label does not provide the consumer with those culture characteristics. Nevertheless, literature value for standard mussel density on ropes cultured in sheltered Galician Rías is between 700 and 1000 mussels·m^{-1} (Irisarri et al. 2014a). Cubillo et al. (2012) observed that mussels cultured at higher densities displayed a lower shell height to shell length ratio compared to mussels cultured at a lower density. Similarly, the author observed that mussels cultured at higher density displayed lower shell width to shell length ratio compared to those cultured at low densities.

Regarding differences in oceanographic conditions, mean current speed in the experimental site was significantly higher than values reported from previous studies carried out in Galician sheltered Rías (Pérez-Camacho et al. 2014a, b). Similarly, highly significant wave height scenarios were also found (with peaks of 796 m) at the experimental site. Indeed, only open ocean mussel growth studies of Buck and Buchholz (2004) and Langan (2000), from the German Bight and the Gulf of Maine, respectively, showed similar wave height scenarios (with peaks of 7.96 m). The mussel shells of the experimental site were characteristically round, wide and great in thickness. Although these observations were not validated through specific comparisons, they are consistent with the findings from previous studies developed with mussels from wave beaten shores (Akester and Martel, 2000, Lewis and Powel 1961, Seed 1968).

Despite the limited information available on the culture method and culture characteristic of commercial mussel product, the new product cultured at the experimental site would compete with current commercial products. No differences regarding mussel appearance of cooked samples were pointed out by the group of local panelists during the sensory test.

In this study, no relevant differences at biochemical composition level other than ash content were observed between the open ocean experimentally cultured mussels and the commercially purchased mussels from Galician Rías. Biochemical composition of mussels is affected by water temperature, nutrient availability, and the reproductive cycle of individuals (Fernández-Reiriz et al. 2007). These multiple factors change seasonally, which, in turn, cause seasonal changes in biochemical composition of mussels (De Zwaan and Zandee 1972; Dare and Edwards 1975; Ruano et al. 2012). As reported by previous authors, major differences in biochemical composition relied on: (i) feeding behavior, in terms of filtration capacity and clearance rate (Vilela 1950; Heral et al. 1980); (ii) quality and quantity of seston available in the environment; (iii) state of sexual maturity of individuals (Fernández–Reiriz et al. 2007).

This study showed similarities on proximate composition between both mussel products, consistent with previous findings from mussels from Galician waters as reported by Fuentes et al. (2009). This may be explained by similarities occurring within the main factors regulating growth and biochemical performance in both culture conditions. Both culture conditions have a close annual average in environmental conditions, the same seasonality in reproductive cycles, and/or the same genetic heritage due to geographic proximity. However, open ocean mussels exhibited a higher amount of ash when compared to commercial ones from Galician Rías. Fuentes et al. (2009) observed similar values of ash content in commercial product from Galician Rías when comparing mussel product from different Spanish regions. Higher ash content than open ocean mussels of this study were also observed in the same study in mussels from Ebro Delta and from Valencia (NE and SE Spain, respectively). Mussels cultured in the exposed environmental conditions (e.g., phytoplankton variability, strong hydrodynamics, and very marked seasonal changes in seston composition) may experience situations of stress or may have greater energetic expenditure (Azpeitia et al. 2016) than mussels cultured in sheltered conditions. Oliveira et al. (2015) found similar results when comparing open ocean mussels to sheltered cultured mussels. The authors related this difference to the hydrodynamic conditions found by mussels in the offshore culture areas, which would interfere with mussel metabolism in a set of complex interactions between temperature, food availability, growth, and reproductive cycle (Gabbot 1976). Thus, ash content is influenced by many abiotic factors such as salinity and presence of metals on the water column (Okumuş and Stirling 1998), as well as by the nutritional state and the reproductive cycle of the animal (Beninger and Lucas 1984). Regarding nutritional state, Mayzaud (1976) associated an increase in ash content of zooplankton with a state of starvation, and Wilkins (1967) observed the same in herrings. Nevertheless, this higher ash percentage may account for the decrease in other constituents (e.g., lipids, proteins) rather than an increase in the amount of ash, as data analysis was performed with relative percentages and not

in absolute terms. Despite these differences in ash content, similar general properties at biochemical level should be expected when comparing the open ocean mussels from the SE Bay of Biscay to their commercial counterparts from another northern Spanish region.

Depending on the time of the year, levels of nutrients, both in Galician Rías and in the open ocean of the SE Bay of Biscay, differ. In Galician Rías, upwelling events typically take place during spring–summer. Highest chlorophyll levels have been recorded during spring (<5 $\mu g \cdot l^{-1}$)(Irisarri et al. 2014a). However, upwelling events are usually followed by thermal stratification periods during the summer which might result in a significant reduction of chlorophyll levels up to 0.4 $\mu g \cdot l^{-1}$ (Irisarri et al. 2014a). The thermocline layer prevents nutrients from fertilizing the surface and chl-a eventually becomes depleted. The author corroborated this with a negative scope for growth (SFG) observed in mussel, when abnormally high temperatures are coupled with aforementioned low energy intake in Galician Rías in summer. In the SE Bay of Biscay, there is also a thermal stratification during summer, which decreases phytoplankton biomass to lower values than those described in Galician Rías (0.2 $\mu g \cdot l^{-1}$ in surface waters) (Morán et al. 2012). However, during summer, short periods of atmospheric instability (storms) may act as modulation factors of this bloom decay in the SE Bay of Biscay. Although inputs of nutrient-rich continental waters can be observed, the intensified aforementioned current velocities may also resuspend inorganic sediments. Thus, in storm conditions, a dilution of available particulate organic matter (POM) for bivalve filter-feeders can be expected (Cranford et al. 2011; Irisarri et al. 2013; Zuñiga et al. 2014) in the experimental site.

The biochemical composition of the present contribution are consistent with previous studies reporting that mussels are high in proteins and low in calories and fat (Shahidi 2004; Fuentes et al. 2009; Gallardi et al. 2014). Indeed, some authors have reported that a daily protein intake of 0.75 g·kg body weight (which means that with 100 g of mussels for a person of 70 kg) is recommended to satisfy approximately 23% of the daily protein requirements (Garcia-Gabarra 2006; Stankovic et al. 2011; James 2013), thus demonstrating the rich nutritional value of mussels for human consumption.

In addition, it is also generally accepted that mussels represent an important source of essential fatty acids and particularly for long-chain polyunsaturated variety (PUFAs) (James 2013). In this study, not only concentration levels but also their representativeness on the profile of the major fatty acid groups (PUFA, MUFA and SAFA) is consistent with previous mussel studies (King et al. 1990; Freites et al. 2002b; Orban et al. 2002). Irisarri et al. (2014b) observed a selective accumulation of PUFA in different tissues of mussels. Mussels have a limited capability for de novo synthesis of PUFAs (Alkalani et al. 2007) and acquire them from diet. The unsaturation degree of the FA increases during the colder seasons, as mussels require these FA to maintain membrane fluidity (Hall et al. 2002). The contrary is observed in warmer seasons and thus, the PUFA level varies in opposite fashion to SAFA level. Mean seawater temperature in open ocean experimental site in summer was slightly higher (Azpeitia et al. 2016) than the typical summer temperature in Galician Rías (Pazos et al. 1997), which could have promoted a faster decrease in the unsaturation degree. This may have caused SAFA level to be higher in experimental mussel than commercial ones. Several authors have reported that higher proportions of SAFA may represent bacterial loads or organic-detritus-rich environments (Galap et al. 1999; Freites et al. 2002b). However, open ocean conditions are related to high hydrodynamic scenarios which favor marine water recycling and consequently less detritus accumulation compared to sheltered sites. Despite this higher water recycling, resuspension of material may have occurred at the experimental site. Moreover, regarding bacterial biomarker FA (15:0, 17:0, 18:1ω7 and 18:1ω7/18:1ω9 > 1; Budge et al. 2001), higher values were observed in mussels from Galician Rías compared to experimental mussels. This may indeed support the aforementioned notion that temperature differences may well lead to higher SAFA levels in experimental mussels.

Major fatty acid levels in marine bivalves are generally known for being highly dependent on biochemical and environmental conditions (Lucas and Beninger 1985; De Moreno et al. 1980; Fernández-Reiriz et al. 1989; Fuentes et al. 2009). Nevertheless, the aforementioned results are consistent with current existing knowledge on mussel biochemistry and could be of use in further research in the same field at a local level.

Palmitic acid (16:0) has been reported in numerous studies (King et al. 1990; Freites et al. 2002a; Orban et al. 2002; Alkalani et al. 2007; Fuentes et al. 2009) as the major saturated fatty acid (SAFA) present in mussels. The experimental open ocean mussels herein displayed not only the same trend, but also, consumers reported a more intense sea-flavor after tasting the product. This would be consistent with Oliveira et al. (2015), who found that palmitic acid along with DHA are mainly responsible for mussel flavour.

PUFA levels of both mussel products herein were abundant and consistent with results from previous mussel studies from other localities (King et al. 1990; Orban et al. 2002; Freites et al. 2002b; Dridi et al. 2007; Alkalani et al. 2007; Fuentes et al. 2009), where healthy mussels always contain PUFA levels near to 50% of the total fatty acids proportion. As reported by Musa-Veloso et al. (2011) a dose of 250 mg·day^{-1} of the long-chain omega-3 fatty acids EPA and DHA (PUFA) is strongly recommended for human health. In this way, although mussels are low in lipids, these lipids are of high quality and provide a healthy source of omega-3 fatty acids with numerous nutritional benefits (Shahidi 2004; Byrd-Bredbenner et al. 2009; James 2013). A high ω6/ω3 ratio has also been reported as harmful to human health (i.e., over values of 12/50; Ackman 1990; Shahidi and Miraliakbari 2004; Fuentes et al. 2009). The results on the EPA and DHA of the present study were suitable within all ranges for both types of mussels examined.

Suspension feeding bivalves feed on living and non-living available material in their habitat (i.e., generalist filter feeders) (Ward and Shumway 2004). They select particles from seston, separating the grain from the chaff, and acquire the optimum energetic budget (Ward and Shumway, 2004). Seston consists of plankton of a wide range of sizes and palatability, material resuspended from the benthos, aggregates, detritus, fecal pellets as well as microorganisms (Ward and Shumway 2004, Karayücel et al. 2010). Fatty acids (FA) have been successfully used as trophic markers of bivalves to identify food sources, habitat preferences, feeding strategies and trophic links (Kharlamenko et al. 1995, 2001; Prato et al. 2010). Several studies on mussel *Mytilus galloprovincilais* Lmk. (Freites et al. 2002a, b; Ezgeta-Balić et al. 2012; Irisarri et al. 2014a) found that variability in nutritional quality of seston was explained by the variance of FAs found in mussels. Bivalves have some capability to synthesize FAs independently of diet (Ventrella et al. 2013), but they are not capable of biosynthesizing de novo polyunsaturated FAs (PUFAs) (Fernández-Reiriz et al. 2011). In addition, fatty acids transferred from primary producers, when incorporated by bivalves, do not undergo major changes (Graeve et al. 1994; Xu and Yang 2007), and thus, fatty acid measurements are a qualitative measurement of both energy transfer and assimilation of nutrients in mussel tissue. FA signature in mussel tissues can reveal the food source of the bivalve (i.e., several weeks prior) (Ezgeta-Balić et al. 2012) and thus, certain FA and their ratios can be used as biomarkers of the diet of the mussel. With these FA ratios, the seasonal contribution of bacteria, phytoplankton classes, as well as microzooplankton to the diet of the mussel can be assessed (Budge et al. 2001; Handå et al. 2012). Such analysis is an important factor in understanding the feeding physiology of mussels (Xu and Yang 2007).

In spite of presenting healthy EPA and DHA proportions, the experimental open ocean mussels of the present study showed less EPA and higher DHA contents than the commercial ones from Galician Rías. EPA level could be related to the ingestion of dinoflagellates (Freites et al. 2002a), whereas DHA could be a consequence of consumption and accumulation of diatoms (Handå et al. 2012). Sidari et al. (1998) observed that *Mytilus galloprovincialis* Lmk. as a preference selected dinoflagellates rather than diatoms. DHA/EPA ratio was >1 in experimental mussels, whereas, it was <1 in commercial mussels. These FA trophic markers (Budge et al. 2001; Dalsgaard et al. 2003) suggest mussels from the experimental site feed on dinoflagellates. Additionally, the ratio 16:1ω7/16:0 was <1 in experimental mussels and >1 in commercial mussels. Thus, all these FA trophic markers suggest commercial mussels feed on diatoms rather than dinoflagellates (Budge et al. 2001; Dalsgaard et al. 2003). No water samples were collected for phytoplankton analysis. However, as described by Álvarez et al. (2008) in the western Iberian Peninsula (i.e., Portugal and Galicia) during spring–summer the continental shelves are strongly affected by upwelling pulses. These natural events are caused by the prevailing winds and the coastline orientation, causing an inflow of deep nutrient-rich waters which boosts phytoplankton productivity (Varela et al. 2005). Moreover, as described by Figueiras et al. (2002) and Álvarez-Salgado et al. (2008), these upwelling pulses result in the transportation of dinoflagellates to the continental shelf and leave the embayment dominated by diatoms. Similarly, Irisarri et al. (2014b) found that diatom FA biomarkers were significantly higher in mussel tissues from Galician Rías during spring–summer although those FA trophic markers were >1 throughout the entire year. Oliveira et al. (2015) also reported higher levels of EPA in mussels (*M. galloprovincialis* Lmk.) from the Galician Rías compared to individuals of the same species but different culture origin [S Atlantic Sea (Portugal)]. The authors suggested that the resultant DHA/EPA ratios would reflect the higher relative ingestion of diatoms. The contribution, however, of phytoplankton (as diatoms and dinoflagellates) to the diet (derived by 16:1ω7/16:0 and 20:5ω3/22:6ω3 ratios) was proportionally low and moderate in open ocean and commercial (*M. galloprovincialis* Lmk.) mussels, respectively. In the present study mussels were harvested in July along the north Spanish coast. There is a high

likelihood that previous poor environmental conditions (Prato et al. 2010) or post-spawning stages (Garmendia et al. 2010; Ortiz-Zarragoitia et al. 2011; Múgica et al. 2015; Cuevas et al. 2015) may have affected mussels' fatty acids composition in summer.

Fatty acid trophic markers of terrestrial organic sources [i.e., $\Sigma(18{:}2\omega6 + 18{:}3\omega3)$] higher than 2.5 were described by Budge and Parrish (1998) as indicative of a terrestrial organic diet source (i.e., terrestrial plants). A significant input of terrestrial markers was observed in the present study, where the experimental open ocean mussels showed to exceed the threshold. This may be related to the aforementioned differences in seston dynamics in Galician Rías and in the SE Bay of Biscay during summer season.

Finally, the presence of 22:2NMID acid was lower in experimental mussels compared to commercial ones. Sánchez-Lazo and Martínez-Pita (2012) also reported the presence of this fatty acid, although accounting for very little proportion.

The sensory analysis herein was consistent with previous mussel studies (Kryzynowek and Wiggin 1979; Ablett et al. 1986; Gökoglu 2002). The panelists were able to determine the mussel product qualitative characteristics on appearance, odor, flavor, texture, and global perception. Both types of mussel (open ocean and commercial) reached scores of 7 over 9 points at hedonic scale for all sensory attributes. In general terms, even though no triangle test was done, the sensory analysis session was performed stably and consistently among panelists.

Regarding appearance and odor, both characteristics were scored slightly lower for the open ocean samples when compared to the commercial ones from Galician Rías. Observed discrepancies on mussel appearance could be related to inherent biological and/or operational factors (e.g., higher variability in the coloration of mussel meat from open ocean conditions; previous industrial procedures applied on the commercial samples, etc.). The results of the present study showed no significant differences in tasting levels between open ocean and commercial products. However, most panelists also identified some distinctive attributes such as intense sea-flavour, softer and more juicy textures, and salty tastes in the open ocean samples.

These results may specifically be related to the absence of previous depuration requirement on the open ocean samples as a result of having an origin with a class A production area according to EU and local public legislation. During depuration processes, mussels are fasted and animals excrete their metabolic waste products, which include salts (Lee et al. 2008). Despite depuration of commercial mussels, the organoleptic characteristics did not differ greatly between both mussel products. The results on texture, at the time of the sensory analysis, also revealed softer meat in experimental mussels. Nevertheless, differences were not statistically significant and thus it can be concluded that softer texture does not imply mussels are less desirable. It is noteworthy that desired quality among fishery products relies on regional preferences, consumers' attitudes and methods of preservation and/or consumption (Haard 1992). Both mussel products reached 7 points out of 9 in qualitative category; a positive result considering the potential of open ocean mussels of the present study to be marketable in the near future. The tested consumers also expressed an intention to purchase both products (>70%). In general terms, this prescreening hedonic analysis showed that experimental open ocean mussels were similar in attributes to those routinely imported from the Galician Rías to the Basque Country. From a marketing perspective, the mussels cultured in the open ocean of the SE Bay of Biscay may be well accepted by consumers and fulfill the minimum requirements to fit and compete in the existing local market. The results of the present study provide baseline information to validate nutritional value characteristics and consumers' acceptance of mussels (*M. galloprovincialis* Lmk.) cultured in the open ocean of the Bay of Biscay. In addition, as a commercial validation was positive, it is now worth investigating how changes in the culture method can optimize production and commercial activity of future aquaculture in the Basque Country.

Conclusion

Space available in coastal areas for aquaculture facilities is growing more and more limited and is subjected to strict jurisdictions and limited leases. In addition, the geography of the coast in many regions might not be sheltered enough for mooring rafts or longlines, while limited seston supply and/or polluted waters can also represent limiting factors for mussel coastal aquaculture. Hence, this study investigates an interesting alternative to traditional suspended mussel culture in coastal inlets.

This study is the first sensory assessment of mussels produced in open ocean from the SE Bay of Biscay. First, this new information will be valuable for the development of strategies that favor origin specifications linked to health benefits which, in turn, would contribute to consumers' decision-making processes (i.e., purchase intention and preferences). Secondly, it will help to predict cost-effectiveness during mussel production and commercialization.

The approach of this study aimed to validate consumer acceptance as a meaningful factor in market feasibility studies. In addition, it also aimed to investigate if mussels cultured in the open ocean could compete with other mussels currently available on the market. However, future market needs will ultimately decide the final viability of mussel open ocean aquaculture.

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