A worldwide reliable indicator to differentiate wild vs. farmed Penaeid shrimps based on 207 fatty acid profiles

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Abstract:

Shrimps and prawns are especially subject to food fraud, which has consequences not only on the economy but also represents a potential risk for public health. Fatty acids (FA) of Penaeid shrimps have been largely explored in the literature, and although they are unable to discriminate shrimps geographical origin or species, they might provide an interesting tool to distinguish their production method (wild vs. farmed). The present study is based on a literature compilation of Penaeid shrimp FA profiles encompassing all continents and 28 species. It reveals that the ratio of FA 18:2ω6 + FA 18:3ω3 / FA 16:1ω7 can differentiate wild vs. farmed Penaeid shrimps with 100% accuracy within the 207 FA profiles of the dataset considered. Assuming a normal distribution of the dataset, 94.4% of the farmed shrimps population is expected to fall above 2.92, and 99.7% of the wild shrimps population is expected to fall below 2.92.

Keywords: seafood, fatty acids, food authenticity, aquaculture, production method, class-modelling analysis

Chemical compounds studied in this article: Myristic acid (PubChem CID: 11005); Palmitic acid (PubChem CID: 985); Stearic acid (PubChem CID: 5281); Palmitoleic acid (PubChem CID: 445638); Oleic acid (PubChem CID: 445639); Linoleic acid (PubChem CID: 5280450); alpha-Linolenic acid (PubChem CID: 5280934); Arachidonic acid (PubChem CID: 444899); Eicosapentaenoic acid (PubChem CID: 446284); Docosahexaenoic acid (PubChem CID: 445580)

1. Introduction

Shrimps and prawns represent the second main group in world trade of fish and fish products in terms of value. The market demand for Penaeid shrimps is steadily growing and both capture fishery and aquaculture production increased in the recent years (FAO 2018). However, benefits and consequences of shrimp consumption are subject to strong debates. On the one hand, presence of high quality proteins, low fat content and fatty acids (FA) with chemopreventive properties makes them healthy products (López-Saiz et al. 2013). On the other hand, expansion and intensification of shrimp farming causes environmental impacts (e.g. destruction of coastal habitats, Richards and Friess 2016) and induce risks for human health (e. g. presence of chemicals or pathogens, Mansfield 2011), while overfishing of low trophic level species leads to ocean depletion (Coll et al. 2008). According to trends in human and environmental concerns within each country, the demand for one or another product may differ, increasing the risks of deliberate mislabelling. Food fraud has consequences not only on the economy but also represents a potential risk for public health (Spink and

Moyer 2011). Thus, reliable analytical methods are needed to authenticate production method, geographical origin and species of food products (Ortea and Gallardo 2015).

Promising methods for shrimp authentication mostly rely on stable isotope and multi-element analysis (Ortea and Gallardo 2015, Gopi et al. 2019). However, to date tools were developed using relatively low amount of data, little species diversity (up to 5 Penaeid species, Ortea and Gallardo 2015) and restricted geographical areas. Strong limitations such as formulated feed composition, seasonal influence on shrimp elemental profiles and data scarcity in the literature make such indicators difficult to generalise at the global scale (Li et al. 2016). As a consequence, to my best knowledge no control organism has yet implemented a method to validate the labelling of production method for Penaeid shrimps. Since Penaeid shrimps are essentially produced in tropical latitudes and developing countries (FAO 2018) and traded over wide geographical areas (Gopi et al. 2019), a study encompassing all continents is required. In addition, although farmed animals massively belong to two species (*Litopenaeus vannamei* and *Penaeus monodon*), shrimps are often sold peeled or headless, making them barely impossible to differentiate from the tens of wild species and leading to frequent mislabelling (Pascoal et al. 2008).

Contrary to stable isotopes and elemental profiling, shrimp FA have been largely explored in previous studies, especially with the objective to improve diet in farmed conditions and to evaluate shrimp nutritional quality for humans. These studies showed that FA were unable to discriminate geographical origin or species of Penaeid shrimps (e. g. Bottino et al. 1980, Yerlikaya et al. 2013) but revealed interesting insights into distinguishing production method. The present study hypothesised that wild and farmed Penaeid shrimps can be differentiated according to their FA profile, regardless of the geographical origin and the species considered. It is based on 8 new profiles from

Vietnam and a literature compilation of 113 FA profiles of wild and 112 profiles of farmed shrimps encompassing all continents and 28 species.

2. Materials and methods

2. 1. Sample collection

Five species of wild shrimps (*Metapenaeus affinis*, *M. brevicornis*, *M. ensis*, *Parapenaeopsis hardwickii* and *Penaeus monodon*) were bought to fishermen in the Can Gio mangrove (Southern Vietnam) during a PhD project studying carbon dynamics and trophic relationships in this ecosystem in 2015. Two species of farmed shrimps were caught from aquaculture ponds and/or bought at the market in Ho Chi Minh City (*Litopenaeus vannamei* and *P. monodon*). Samples were brought in the lab on ice and stored at -25°C for further analysis of FA.

2. 2. Fatty acids analysis

Shrimp shells were removed and tail muscle was freeze-dried and powdered before lipid extraction. Individual samples consisted in one shrimp tail and three to four samples were analysed for each of the eight combinations of species, sampling area and production method (see Supplementary Material for details). Lipids were extracted with a mixture of chloroform:methanol:water (1:2:1, v/v/v) following a slightly modified protocol of Bligh and Dyer (1959), as described in Meziane et al. (2007). Fatty acids were methylated using a boron trifluoride-methanol solution and fatty acid methyl esters were quantified with a GC-FID (Varian 3800-GC). Fatty acid identification was performed using a GC-MS (Varian 450-GC; Varian 220-MS) and comparison of GC retention times with commercial standards. Fatty acid proportions were reported as % of total FA (Supplementary Material).

2. 3. Literature review

The Web of Science database was crawled in November 2018 using the search string “wild” AND “fatty acid” AND “shrimp” OR “prawn”. Among the 105 returned results, 7 studies reported relevant FA profiles of wild and/or farmed Penaeid shrimps. Cited and citing literature of these 7 papers were then explored to seek for supplementary data, resulting in 32 additional studies reporting Penaeid shrimp FA profiles. In total, 201 profiles published between 1975 and 2018 could be obtained using this methodology. A lot of data available in the literature was necessarily ignored given the hundreds of papers on shrimp and prawn FA compositions, mainly dealing with aquaculture topics. This choice provided a random method of choosing among available FA profiles and constructed a large enough database for the purpose of this work. After a first data analysis, three additional studies conducted for aquaculture purpose (25 additional FA profiles of farmed shrimps) and providing both the FA composition of shrimp food and shrimp muscle were added to the database to challenge the robustness of the indicator. These studies reported notably high levels of the FA 16:1ω7 in shrimp food, thus allowing to measuring its inclusion into muscles. Adding the 8 new profiles of this study, the database consisted in 118 FA profiles of wild shrimps and 116 profiles of farmed shrimps, distributed in 28 species (5 species for farmed shrimps) and spread over all continents (Fig. 1). When the authors did not precisely indicate geographical origin of shrimps, the location of the study province capital city was recorded and when mixed samples were used, the first mentioned site was used. Locations were slightly moved when necessary (max 3° in latitude and/or 6° in longitude) to avoid overlay in Fig. 1.

Average proportions of all identified FA provided by the authors and study details are reported in Supplementary Material. The authors mainly employed slightly modified versions of the Bligh and Dyer (1959), Folch (1957), Metcalfe (1966) and Lepage and Roy (1986) protocols for lipid extraction and all were used for both the analysis of wild and farmed shrimps (Supplementary Material). In addition, 9 studies (including this one) among the 43 considered studied both wild and farmed shrimps using the same fatty acid analysis protocol, thus dismissing a bias that could be induced by lipid extraction method. Species names were modified when necessary to reflect recent changes in taxonomy and comply with valid names provided by the World Register of Marine Species database. Only muscle FA were considered, except for post larvae studies where FA were generally those of the whole shrimp. For a few studies, only polar lipids were quantified. Since they are major components of shrimp muscle FA (O'Leary and Matthews 1990) they were considered similarly as profiles of total FA.

2.4. Statistical analysis

A few adaptations had to be made to make FA profiles comparable to one another. First, only FA quantified in at least 80% of profiles were considered, leading to a list of 10 FA for those proportions were recalculated to reach 100% (Table 1). When the authors reported only the number of unsaturations of a given FA but not their first position, it was converted to the generally most abundant corresponding FA. Thus, FA 16:1 was converted to 16:1ω7, 18:1 to 18:1ω9, 18:2 to 18:2ω6, 18:3 to 18:3ω3, 20:5 to 20:5ω3, 20:4 to 20:4ω6 and 22:6 to 22:6ω3. Some authors differentiated -cis and -trans forms of a given FA. Since it was not possible to know whether others authors grouped both forms in their results or provided only the -cis form, which was the most abundant, both were summed. This choice actually had poor influence on results given that the -

trans form of a given FA was always present in negligible proportions compared to the -cis form. Finally, in one study, a high proportion of FA 16:1 was reported as 16:1ω9 and FA 16:1ω7 was not detected (Chanmugam et al. 1983). This was considered as a mistake and 16:1ω9 was converted to 16:1ω7.

Global differences between FA profiles were visualised using non-metric multidimensional scaling (NMDS) based on a Bray-Curtiss distance matrix. Significant differences of a given FA in wild vs. farmed Penaeid shrimps were revealed using Student test on square root transformed data to alleviate heteroscedasticity. The threshold value of the FA indicator was calculated using univariate linear discriminant analysis (LDA). The robustness of the indicator was challenged by repeating the calculation 1000 times using a random subsample of 80% of the dataset. Finally, probability predictions were calculated based on mean and standard deviations of both groups according to normal distribution laws. LDA and probability predictions were calculated on log-transformed data to alleviate heteroscedasticity.

3. Results and discussion

3. 1. Ability of FA to differentiate wild vs. farmed shrimps

The NMDS based on the 10 most commonly quantified FA shows a clear differentiation between FA profiles of wild vs. farmed Penaeid shrimps (Fig. 2), except for one profile of wild shrimp (study n° 27, Zhou et al. 2017). For routine testing purposes, it is poorly convenient to position a FA profile to be assessed in a previously built multivariate analysis. A more suitable indicator would be to use the proportion of a single FA or a group of FA, or even the ratio between singles or groups of FA, thus avoiding a bias induced by the number of FA detected.

Given the amount of data in this study, the Student tests detected differences between almost all FA proportions of wild vs. farmed shrimps (Table 1). However, lowest overlays between wild and farmed profiles (<5% of data) were measured for FA 16:1ω7 and C_{18} PUFA (18:2ω6 + 18:3ω3). Proportions of the FA 16:1ω7 were higher in wild shrimps compared to farmed shrimps, and the opposite was measured for C_{18} PUFA proportions (Table 1). A few profiles would nevertheless fall in the wrong category if only one or the other FA was considered (Fig. 3a and 3b). The ratio of C_{18} PUFA on 16:1ω7 segregated the few remaining overlaying data and provided a reliable indicator to differentiate wild vs. farmed Penaeid shrimps whatever their geographical origin (Fig. 3c). Null proportion of FA 16:1ω7 excluded 26 FA profiles from this ratio, mostly from studies conducted for aquaculture purpose and where authors decided to present only major FA (e.g. Wouters et al. 1997, Gonzalez-Felix et al. 2010). These samples most probably exhibited low levels of FA 16:1ω7, making them fall in the expected category. One exception is the study of Menard et al. (2015) whose purpose was to compare shrimps of wild and farmed origin but who did not mention the FA 16:1ω7. Finally, only one profile already highlighted by the NMDS (study n° 27; Fig. 2) did not fall in the expected category. The authors measured especially low levels of FA 16:1ω7 and high levels of C_{18} PUFA in their samples (1.98 and 13.09 %, respectively; Zhou et al. 2017), which seems more characteristic of farmed shrimps (Table 1). The study of Zhou et al. (2017) was conducted in order to improve lipid extraction process, not to compare wild vs. farmed shrimps, and the authors bought their samples in a local fish market in Jiangsu province (China). In the nearby province of Zhejiang, Li et al. (2011) analysed farmed individuals of the same species, *Penaeus chinensis*, and obtained similar levels of FA 16:1ω7 and C_{18} PUFA (1.2 and 13.6 %, respectively) than those of Zhou et al. (2017). It is thus highly probable that the products studied by Zhou et al. (2017) were

mislabelled and were actually of farmed origin. The corresponding profile was removed to calculate probability predictions.

Maximum value of the C_{18} PUFA / FA 16:1ω7 ratio for wild shrimps was 2.33 and minimum value for farmed shrimps was 3.18 (Table 1). The threshold between wild and farmed shrimps was set to 2.92 by the LDA, considering 91 data of wild shrimps and 116 of farmed shrimps. When 80% of randomly selected data was used, the threshold ranged from 2.74 to 3.17 in 99% of cases, thus always allowing a correct classification of the entire dataset. However, although the value of the threshold is robust in the present case, control organisms may want to employ a class-modelling analysis instead of a discriminant analysis to implement incertitude boundaries and minimise false-positives (Lopez et al. 2015). According to the distribution of farmed shrimps dataset (log-transformed to increase normality), 94.4% of the population is expected to fall above 2.92. The risk of incertitude can be reduced to 1% (or 0.1%) by not adjudicating farmed shrimps with a ratio between 1.30 (or 0.56) and 2.92. Similarly, 99.7% of wild shrimps population is expected to fall below 2.92. Reducing the risk to 0.1% would lead not to adjudicate shrimps with a ratio between 3.79 and 2.92. Reducing false-positives probability to 1% (0.1%) would have led 9 (22) profiles of wild shrimps among 91 and 0 (2) profiles of farmed shrimps among 116 in this study to be classified as doubtful.

Studies reported in the database were conducted to evaluate the effect of season (e. g. n° 16, Bottino et al. 1980), shrimp size (e.g. n° 7, Bragagnolo and Rodriguez-Amaya 2001), shrimp species (e. g. n° 16, Bottino et al. 1980), shrimp sex (e. g. n° 36, Dincer and Aydin 2014), catching depth (e. g. n° 31, Yerlikaya et al. 2013), drying methods (e. g. n° 39, Akintola et al. 2013) and cooking methods (e. g. n° 30, Delfieh et al. 2013) on wild shrimp FA profiles. It included a high diversity of variability sources, strengthening the applicability of the proposed indicator. Similarly, farmed shrimps were studied to assess

the effect of lipid source (e. g. n°42, Gonzalez-Félix et al. 2002), lipid levels (e. g. n° 34, Ouraji et al. 2010), fishmeal replacement (e. g. n° 26, Panini et al. 2017), phospholipid addition (e. g. n°42, Gonzalez-Félix et al. 2002) and probiotic administration (e. g. n° 22, Ramezani-Fard et al. 2014) on their FA compositions. Bottino et al. (1980) suggested that diet was the main factor influencing composition of shrimp body lipids and that shrimp species and endogenous adjustments to external conditions had lower effect. However, given the diversity of diets that has been tested in farmed conditions, it is poorly probable that the only deposition of ingested FA in lipids never led to overlays with FA profiles of wild shrimps, as suggests the indicator proposed in this study.

Table 1: Average ± SD (5^{th} – 95^{th} centile) proportion of the 10 most frequently quantified FA in wild and farmed Penaeid shrimps

Fatty acids (%)	Wild shrimps (n = 118)	Farmed shrimps (n = 115)	p-value
14:0	2.3 ± 2.0 (0.0 - 7.7)	0.8 ± 1.0 (0.0 - 3.0)	***
16:0	20.7 ± 3.8 (16.2 - 27.1)	21.0 ± 3.2 (15.7 - 25.8)	
18:0	12.1 ± 3.3 (6.0 - 15.9)	12.2 ± 2.1 (8.6 - 15.1)	
16:1ω7	8.2 ± 2.4 (4.7 - 12.5)	1.3 ± 1.2 (0.0 - 4.1)	***
18:1ω9	14.0 ± 4.4 (8.5 - 23.9)	18.8 ± 5.9 (12.4 - 29.1)	***
18:2ω6	2.6 ± 1.9 (1.2 - 6.9)	18.6 ± 9.2 (6.4 - 35.9)	***
18:3ω3	1.0 ± 2.3 (0.0 - 2.3)	4.3 ± 5.4 (0.5 - 17.9)	***
20:4ω6	8.1 ± 4.1 (0.6 - 14.9)	3.5 ± 2.1 (0.9 - 6.4)	***
20:5ω3	16.9 ± 3.1 (12.2 - 21.8)	11.3 ± 4.2 (5.4 - 18.4)	***
22:6ω3	14.1 ± 3.8 (9.1 - 19.0)	8.3 ± 4.3 (2.4 - 15.3)	***
∑SFA	35.1 ± 5.5 (28.3 - 45.4)	34.0 ± 4.3 (26.2 - 40.3)	
∑PUFA	42.7 ± 6.9 (30.2 - 52.6)	46.0 ± 5.7 (36.3 - 53.8)	***
$\sum C_{18}$ PUFA	3.6 ± 3.3 (1.3 - 10.0)	22.8 ± 9.2 (11.2 - 40.5)	***
∑HUFA	39.1 ± 7.1 (27.2 - 49.7)	23.1 ± 8.6 (9.7 - 36.1)	***
ω3/ω6	0.3 ± 0.2 (0.1 - 0.7)	1.1 ± 0.9 (0.3 - 3.1)	***
$\sum C_{18}$ PUFA / FA 16:1ω7[α]	0.52 ± 0.74 (0.02 - 2.33)	32.25 ± 41.73 (3.18 - 159.25)	***

p-value refers to Student test significance: *** $p<0.001$
Σ SFA 14:0 + 16:0 + 18:0
Σ PUFA 18:2ω6 + 18:3ω3 + 20:4ω6 + 20:5ω3 + 22:6ω3
Σ HUFA 20:4ω6 + 20:5ω3 + 22:6ω3
[α] Due to missing values and exclusion of Zhou et al.'s (2017) study, n_{wild} = 91 and n_{farmed} = 116. Values in parenthesis refers here to complete range (min - max). Student test was performed on log-transformed data instead of square root transformed data

3. 2. Origin of FA differences between wild and farmed shrimps

Main differences in FA proportions of wild vs. farmed shrimps were measured in FA 18:2ω6, 18:3ω3 and 16:1ω7 (Table 1). The large abundance of C_{18} PUFA in farmed shrimps has been previously reported in studies comparing shrimp origin (Bottino et al. 1980, Montano and Navarro 1996) and attributed to the inclusion of vegetal oils such as soybean oil (rich in 18:2ω6) or linseed oil (rich in 18:3ω3) in shrimp pellets (Gonzalez-Felix et al. 2002). Actually, there is a clear correlation between proportions of both FA in shrimp diet and in shrimp muscle (Fig. 4a and 4b). Shrimps have little ability to elongate FA 18:2ω6 into 20:4ω6 and FA 18:3ω3 into 20:5ω3 and 22:6ω3 (Bottino et al. 1980). Thus, C_{18} PUFA are being accumulated in farmed shrimp tissues, while HUFA are generally depleted compared to wild shrimps (Table 1).

The higher proportions of FA 16:1ω7 in wild shrimps compared to their farmed counterparts were however much less discussed. Although a few authors actually noticed such differences (O'Leary and Matthews 1990, Montano and Navarro 1996), none have explained underlying reasons for this. At least three causes may lead to lower proportions of FA 16:1ω7 in wild shrimp lipids, but partitioning them would require further investigations. First, in the wild, shrimps are expected to obtain FA 16:1ω7 by the direct consumption of diatoms or eustigmatophyceae, whose FA 16:1ω7 constitutes 23-27 % of total FA (Dalsgaard et al. 2003), or through intermediate trophic level species (e. g. copepods) themselves consuming these microalgae (Willems et al. 2016). Farmed shrimps receive lower proportions of FA 16:1ω7 (up to 9.5%) but unlike C_{18} PUFA, the proportion of FA 16:1ω7 in shrimp muscles does not seem to correlate with that of shrimp food and generally remains below 4% (Fig. 4c), suggesting that other reasons than diet may maintain low FA 16:1ω7 in shrimp tissues. This hypothesis would however require feeding trial to be confirmed since proportion range of FA 16:1ω7 in

diet of existing studies remained much lower than that of 18:2ω6, that sometimes exceeded 50% (Fig. 4a). Second, different lipid class distribution and inequalities in the repartition of FA 16:1ω7 in lipid categories could contribute to differences between wild and farmed shrimps. O'Leary and Matthews (1990) actually measured higher proportions of triglycerides and lower proportions of phospholipids in farmed *P. monodon* compared to their wild counterparts, while Johnston et al. (1983) detected higher proportions of FA 16:1 in phospholipids of *P. aztecus* compared to triglycerides. Again, these differences seem too low to explain alone the on average 7 times higher proportion of FA 16:1ω7 in wild shrimps compared to their farmed counterparts (Table 1). Third, findings from teleost revealed that FA 16:1ω7 was preferentially utilised during β-oxidation (Sidell et al. 1995), the major catabolic pathway of FA in shrimps (Chang and O'Connor 1983). The study of Sidell et al. (1995) involved the enzyme carnitine palmitoyltransferase, whose gene encoding expression was enhanced with dietary plant oil intake in the spiny lobster *Sagmariasus verreauxi* (Shu-Chien et al. 2017). Formulated feed most probably always contain higher levels of plant-derived lipids than natural diet of shrimps, thus increasing β-oxidation and lowering the relative deposition of FA 16:1ω7 in farmed shrimp muscles compared to their wild counterparts.

4. Conclusion

The ratio of C_{18} PUFA (18:2ω6 + 18:3ω3) on 16:1ω7 allows a clear differentiation between wild and farmed Penaeid shrimps for the large majority of consumed species and whatever their geographical origin, thus confirming the hypothesis stated in the introduction. This ratio is most generally below 2.92 in wild shrimps and above 2.92 in their farmed counterparts. Although high levels of C_{18} PUFA were commonly measured in farmed shrimps and attributed to the inclusion of vegetal oils in shrimp food, FA

16:1ω7 has received less attention. Exploring proportions of this FA in wild vs. farmed shrimps allowed the correct classification of the few FA profiles that would be incorrectly labelled using C_{18} PUFA proportion alone. The underlying control of FA 16:1ω7 in shrimp muscles is most probably controlled by at least three factors (diet, lipid class distribution and selective β-oxidation). Further researches would be needed to understand respective contributions of these three factors on overall proportions of FA 16:1ω7.

Appendix

Supplementary data associated with this article is provided as an Excel file named "Supplementary Material.xlsx".

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Figure captions

Fig. 1: World map representing the origin of shrimps for those FA profiles were compiled in this study. Number in the bubbles refers to the reference paper as listed in Supplementary Material

Fig. 2: NMDS of Penaeid shrimp FA profiles based on relative proportions of the 10 most commonly quantified FA. Number in the bubbles refers to the reference paper as listed in Supplementary Material

Fig. 3: Proportions of **a** C_{18} PUFA, **b** FA 16:1ω7 and ratio of **c** C_{18} PUFA/FA 16:1ω7 in muscles of Penaeid shrimps from wild and farmed origin. Note that vertical axis of part **c** was log-transformed to improve data visualisation. Number in the bubbles refers to the reference paper as listed in Supplementary Material.

Fig. 4: Proportions of FA **a** 18:2ω6, **b** 18:3ω3 and **c** 16:1ω7 in shrimp muscles as a function of their proportions in shrimp diet. Dot line corresponds to the 1:1 relationship. Number in the bubbles refers to the reference paper as listed in Supplementary Material

Highlights

- The ratio C_{18} PUFA / FA 16:1ω7 differentiates wild vs. farmed Penaeid shrimps
- Wild Penaeid shrimps with a ratio above 2.92 can be qualified as mislabelled
- Farmed shrimps between 1.30 and 2.92 are doubtful and those below 1.30 mislabelled
- The indicator can be used whatever the geographical origin or species of Penaeid
- Differences in FA are due to both feeding resources and induced metabolic responses