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Suitability of instrumental analysis for the discrimination between wild-caught and conventionally and organically farmed shrimps

Ute Ostermeyer · Joachim Molkentin · Ines Lehmann · Hartmut Rehbein · Hans-Georg Walte

Abstract Shrimps, primarily *Penaeus monodon* and *Litopenaeus vannamei*, from organic and conventional farms and free-living stocks were purchased from the German market over 1 year. This study examined the applicability of established analytical methods for the confirmation of the correct labelling of shrimp products. After species identification of 77 shrimp products, the proximate composition, carotenoid pattern, fatty acid profile and stable isotopes of carbon and nitrogen in the lipids and/or the defatted dry matter (DDM) were determined. To differentiate between the three types of production (wild, organically farmed or conventionally farmed), parameters alone or in combination, partly derived by multivariate tests, were considered. Stable isotope ratio mass spectrometry allowed the differentiation between organically and conventionally farmed *Litopenaeus vannamei* using the combination of $\Delta\delta^{13}C$ and $\delta^{15}N_{DDM}$ values. The gas chromatographic analysis of fatty acids also distinguished between organically and conventionally farmed shrimp of this species. The ratio of the free astaxanthin configurational isomers in shrimp flesh, analysed by high-performance liquid chromatography (HPLC), was inadequate for any assignment, because of the apparent ability to alter the structure of the ingested carotenoids. Thus, a general differentiation of the three production types, irrespective of individual species, could not be achieved by any single method.

Keywords Stable isotopes · Fatty acids · Astaxanthin · Species identification · Partial least-squares discriminant analysis · Organically farmed shrimps

U. Ostermeyer · I. Lehmann · H. Rehbein
Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institute (MRI), Federal Research Institute of Nutrition and Food, Palmaille 9, 22767 Hamburg, Germany
e-mail: ute.ostermeyer@mri.bund.de

J. Molkentin · H.-G. Walte
Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institute (MRI), Federal Research Institute of Nutrition and Food, Herrmann-Weigmann-Str. 1, 24103 Kiel, Germany

Introduction

Warm water shrimp are of great economic importance for aquaculture and fisheries in tropical and subtropical areas. They represent approximately 80 % of the global shrimp market [1]. *Penaeus monodon* and *Litopenaeus vannamei* are two species that dominate the market.

Penaeus monodon (P. monodon), also known as the giant black tiger prawn, is brown in colour with dark brown, black or blue ribbons and reaches 35 cm in length and 150 g in weight. Their natural habitats are the tropical coastal waters of the Indian and Pacific Oceans near Australia, South Asia and East Africa. *P. monodon* grows rapidly and tolerates different salinities but is more susceptible to disease. In aquaculture, wild-caught individuals are often used for breeding. *P. monodon* eat almost any organic substance but prefer protein-rich animal feed, as it is the most carnivorous *Penaeus* species. According to the Food and Agriculture Organization of the United Nations (FAO) statistics (Fig. 1) [2], approximately three times as many *P. monodon* are produced via aquaculture compared to wild-caught shrimp of the same species.

Litopenaeus vannamei (L. vannamei), also known as the whiteleg shrimp, Pacific white shrimp or white tiger, reaches 23 cm in length and is translucent white in colour or occasionally slightly bluish. Wild *L. vannamei* are only found in the tropical waters off the Pacific coast of Central

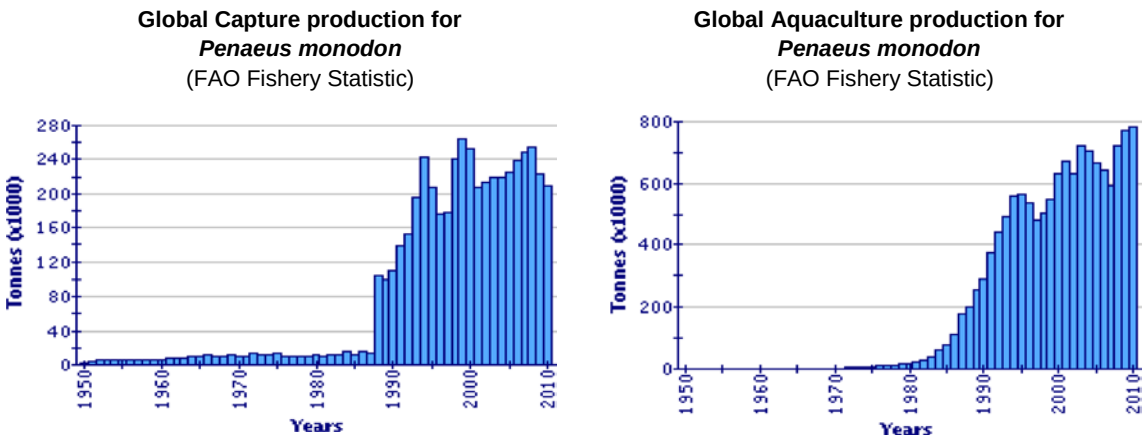

Fig. 1 Global wild-capture and aquaculture production of *P. monodon* [2]

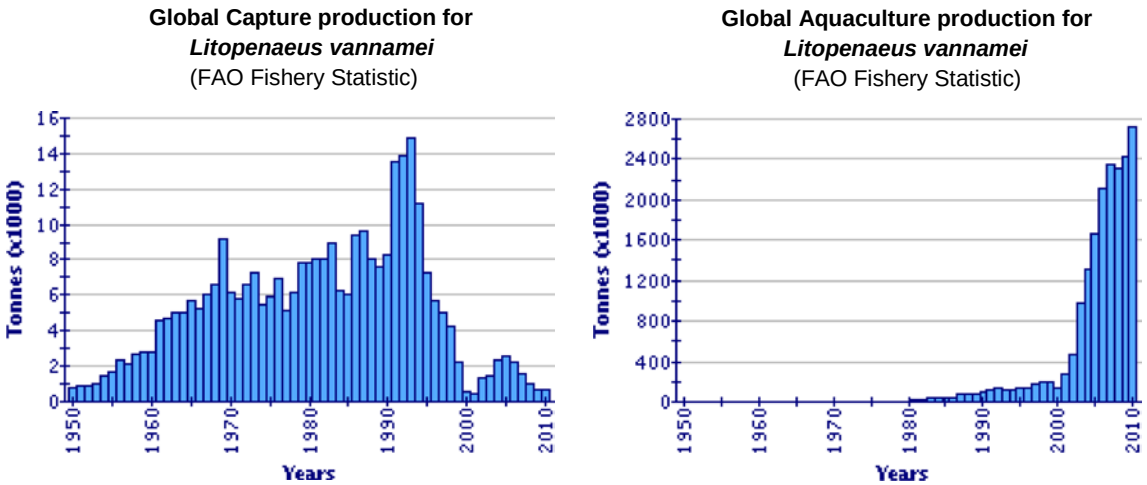

Fig. 2 Global wild-capture and aquaculture production of *L. vannamei* [2]

and South America. This shrimp species is relatively robust and, therefore, is one of the most important species in aquaculture. *L. vannamei* has better survival than *P. monodon* and other Penaeidae shrimps in aquaculture and is relatively inexpensive to raise, due to lower nutritional needs, including lower protein requirements. White shrimp eat a wide variety of food: worms, clams, other crustaceans, algae, phytoplankton and other organic substances. Although *L. vannamei* is a marine shrimp, it also tolerates water of low salt content (7–34 ‰). In addition, this species is easy to breed in captivity and is more tolerant of poor water quality than *P. monodon*. According to FAO statistics (Fig. 2) [2], this shrimp species is commercially sold almost exclusively as farmed shrimp.

The quantity of *L. vannamei* produced worldwide in nearly 30 countries is approximately three times as large as that of *P. monodon*. While *P. monodon* is farmed almost exclusively in the coastal area, *L. vannamei* can also be produced inland. Recently, in Thailand and Indonesia, many farms of *P. monodon* have been converted to *L. vannamei* [3]. Since 2000, a multitude of new farms were established, especially in China. With an annual production of more than 1 million tonnes, China is now the largest producer of *L. vannamei* [4]. At present, this shrimp has the highest worldwide production in aquaculture of all shrimp species [5].

Conventionally farmed shrimp have a poor image among many consumers. Shrimp production is associated with negative social and ecological effects. It has been documented that shrimp have been farmed under poor hygienic conditions and are loaded with chemicals and drugs [6–8]; these conditions encourage organic shrimp farming. The first organic shrimp were produced in Ecuador in agreement with the private standards of Naturland [9] and were imported to Europe in 2001. Currently, the European Commission Regulation (EC) No 710/2009 provides general

rules for aquaculture, including a section specific to shrimp farming (Annex XIII a, Section 7) [10]. Today, organic shrimp are produced in Ecuador, Peru, Brazil, Costa Rica, Vietnam, Thailand, Bangladesh and Indonesia and are most often certified by Naturland. Globally, less than one per cent of the area used for shrimp aquaculture is certified organic [11]. Organically farmed shrimp is significantly more expensive than conventional shrimp.

Shrimp are mainly omnivorous organisms with several microorganisms, aquatic invertebrates and algae serving as their natural feed. While extensively managed shrimp farms primarily rely on the natural productivity of the ponds for food sources, intensively managed farms are dependent on shrimp feeds, either exclusively or in addition to naturally occurring sources. Conventional shrimp feeds often contain fishmeal and fish oil plus a vegetable source of carbohydrates, such as wheat, corn, rice or cassava [12]. Other products such as the astaxanthin-containing meals of crustacean, krill, mussel or shrimp may also be used [13].

According to Naturland standards for organic aquaculture [14], the amount of external shrimp feed should be kept as low as possible. In this case, the shrimp are encouraged to use natural food sources (phytoplankton, zooplankton) present in the ponds. Furthermore, the fishmeal and total protein content of the diet should be reduced as much as possible. Provisional maximum contents are 20 % for fishmeal or -oil and 30 % for total protein. Shrimp companies certified after the enforcement of the EU regulation (EC) 710/2009 are allowed to add fishmeal to the feed only up to an amount of 10 % [10].

Several studies have shown that the fatty acid composition of shrimp flesh is greatly influenced by their diet. The comparison of the fatty acid profiles of *Fenneropenaeus indicus* and *L. vannamei* has already indicated a distinction between wild and farmed shrimp [15, 16].

The stable isotope composition of animals is also significantly influenced by their diet. The isotope ratios of carbon and nitrogen measured by isotope ratio mass spectrometry (IRMS) are particularly suitable for the analysis of feeding effects on animal products. For example, animal protein generally has higher $\delta^{15}N$ values than vegetable protein. The different origins of feed components have resulted in measurable deviations in the tissues of fish. In rainbow trout, the $\delta^{13}C$ and $\delta^{15}N$ values of muscle tissue were significantly higher after continuous feeding of animal protein than of plant protein [17]. The differentiation of wild and conventionally farmed fish by IRMS, partly in combination with the analysis of fatty acids, has been well-described [e.g. 18–26].

Carotenoids, responsible for the colouration of many fish and crustaceans in the wild, are only produced by plants, yeasts, algae and bacteria. Shrimp like other animals are unable to synthesise carotenoids *de novo*; however, after absorption from their diet, the carotenoids can be deposited in their body either in the native form or as metabolites [27]. Shrimps such as *P. monodon* and *L. vannamei* have the ability to alter β-carotene, zeaxanthin and canthaxanthin to astaxanthin [28, 29].

Wild shrimp eat large quantities of microalgae, which are the primary producers of astaxanthin in the sea. Therefore, astaxanthin is the main carotenoid in shrimp [30]. Here, it exists either in the free form, esterified with one or two fatty acids or attached to macromolecules, especially proteins. In the soft tissues of shrimp, carotenoids are present mainly in the esterified form, while in the cephalothorax and the abdominal exoskeleton a coloured protein-astaxanthin complex is also present, which dissociates during cooking resulting in the orange-red colour of free astaxanthin [27–29]. Free-living shrimp are more strongly coloured than farmed shrimp because of the higher amount of astaxanthin. However, this content can vary depending on the season, as marine algae are subject to seasonal fluctuations [31].

The astaxanthin molecule has two chiral centres, located at the C3 and C3′positions, which yield three configurational isomers: a pair of enantiomers (3S,3′S) and (3R,3′R) and a *meso* form (3R,3′S). The ratio of these isomers (3R,3′R) : *meso* : (3S,3′S) depends on their origin; there are large differences between the isomer ratios of synthetic (1:2:1) and natural astaxanthin; for example, in the yeast *Phaffia rhodozyma* (predominant 3R,3′R), the bacterium *Paracoccus carotinifaciens*, the microalgae *Haematococcus pluvialis* (both predominant 3S,3′S) and the Antarctic krill (70:21:9) [32, 33]. According to the European Regulation (EC) No 1831/2003 the use of astaxanthin and canthaxanthin as feed additives is only permitted for salmon and trout [34]. Naturland permits the use of natural pigment supplements in the form of shrimp shells or *Phaffia* yeast in organic aquaculture [14]; however, the SOIL Association's organic standards do not permit the use of these two ingredients in shrimp feed [35].

In wild *P. monodon*, *L. vannamei* and other Penaeidae species, the (3R,3′R), *meso* and (3S,3′S) astaxanthin isomers were found in a ratio of approximately 1:2:2 [30]. In vivo racemisation of astaxanthin was observed in *Penaeus japonicus* [36].

Due to the increasing demand for organically produced fishery products and their higher prices, there is a potential risk of false declaration of conventional products as organic goods. To protect the consumer against misleading and deception, as well as to protect the organic producer, tools are required to allow verification of organically farmed shrimp. Methods are necessary that permit the differentiation between conventionally and organically farmed as well as wild-caught shrimps. Currently, this distinction has not been examined. In the present study, the suitability of

Table 1 Origin of the shrimp samples

Production method	Number of products	Species	Origin
Wild	7	P. monodon	FAO 34 (Atlantic, Eastern Central) FAO 71 (Pacific, Western Central)
Wild	18	Metapenaeus spp., Fenneropenaeus spp.	FAO 51 (Indian Ocean, Western) FAO 57 (Indian Ocean, Eastern) Pacific
Organic	11	P. monodon	Bangladesh, Vietnam
Organic	14	L. vannamei	Costa Rica, Ecuador
Conventional	14	P. monodon	Bangladesh, Vietnam
Conventional	13	L. vannamei	China, Indonesia, Thailand, Vietnam

the established methods of analysing stable isotopes, fatty acids and carotenoids was evaluated by examining shrimp products of different origin, including retail products.

Materials and methods

Samples

To account for company-specific variations, shrimp from several organic and conventional farms were purchased. The variation in the composition of individual animals and seasonal fluctuations were accounted for by examining several individuals of each farm and by sampling at multiple times during the year. Only the edible parts of the shrimp samples were used for analysis.

Shrimp samples (77) were purchased deep-frozen from wholesale and supermarkets between 4/2011 and 2/2012 (Table 1). The package sizes varied between 150 g and 1 kg. If packages had a net weight of less than 500 g, then at least 2–4 packages of a product with the same batch number were purchased and a homogenised, composite sample was produced. To identify the species, 3–5 frozen shrimp tails or whole animals of each composite sample were analysed. All shrimp samples were raw (one exception) and were bought as whole, headless or peeled animals.

Organic shrimp samples (25) were from Bangladesh, Vietnam, Ecuador and Costa Rica. They were purchased from eight different producers. If a certifier was indicated, the products were certified by Naturland.

Conventionally farmed shrimp samples (27) produced by 12 companies were analysed. Bangladesh, Vietnam, Thailand, Indonesia and China were indicated as the countries of origin. Many of the conventionally produced products contained additives such as citric acid, citrates, sodium bicarbonate, sulphite, phosphate (E 450-E 452) and potassium tartrate.

Since a sampling of shrimp on site has not been possible, the assignment of the type of production had to be based on the product declaration.

In addition, 25 wild shrimp samples were examined, which originated in the FAO fishing areas 34, 51, 57 and 71 (http://www.fao.org/fishery/area/search/en). Most samples were labelled as *Penaeus* spp. Citric acid and sulphite had been added to some wild shrimp samples during production. The products were from six different manufacturers.

All samples were stored at −40 °C until analysed.

Proximate analyses

Moisture content

The moisture content was determined gravimetrically after approximately 5 g of the homogenate blended with sea sand was dried for 18 h at 105 °C.

Protein content

Protein nitrogen was measured with a LECO TruSpecN (LECO Instruments GmbH, Mönchengladbach, Germany). The determination based on the principles of the Dumas method [37]. The total protein content was calculated by multiplying the per cent nitrogen content by 6.25 [38].

Lipid content

The determination of the lipid content was carried out according to a modified method of Smedes [39]. Total lipids were extracted from the homogenised sample with a mixture of isopropanol and cyclohexane. After addition of water, the lipid-containing organic phase was separated, evaporated, dried and weighed.

Species identification by PCR-based DNA analysis

A segment of the mitochondrial 16S rRNA gene with 312 base pairs was amplified, analysed by SSCP (SSCP = Single Strand Conformation Polymorphism) and sequenced [40]. The obtained sequences were compared to each other and to sequences in GenBank (http://www.ncbi.nlm.nih.gov) using BLAST (Basic Local Assignment Search Tool) analysis.

Gas chromatography of fatty acids

Fatty acid methyl esters (FAME) were obtained from the extracted lipids by transesterification with potassium hydroxide [41]. Fatty acid composition was determined according to the DGF standard method with gas chromatography (GC-FID) [42]. Analyses were performed on an Agilent 7890 gas chromatograph (Agilent Technologies) equipped with split injection port, autosampler, FID and a 60-m fused silica capillary column (i.d.: 0.32 mm) coated with 0.25 µm of DB-23 (Agilent J&W). Internal standard: Nonadecanoic acid C19:0. Fatty acids in the range of 14:0–22:6 n-3 were measured and represented as a per cent of all measured fatty acids.

Isotope ratio mass spectrometry (IRMS)

The lipids (LIP) were extracted from 12.5 g of shrimp homogenate based on the method of Smedes [43]. The remaining aqueous phase together with sediment was freeze-dried to obtain the defatted dry matter (DDM). The analysis included the stable isotopes of carbon ($\delta^{13}C$) in lipids and DDM and nitrogen ($\delta^{15}N$) in DDM. For the determination, 0.75 mg DDM or 0.46 mg lipids were weighed into tin capsules and combusted in the Flash EA 1112 elemental analyser (Thermo Scientific, Waltham, MA). The resulting CO_2 and N_2 were analysed by isotope ratio mass spectrometry (IRMS).

The stable isotope analysis of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) was performed using a Delta^{plus} XL isotope ratio mass spectrometer (Thermo Scientific) with the software ISODAT 2.0 (Thermo Scientific). The isotope ratios are given in ‰ on a δ-scale and relate to the international standards VPDB and AIR. The $\delta^{13}C$ values are calculated as follows ($\delta^{15}N$ accordingly):

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1{,}000; \quad R = {}^{13}C/{}^{12}C$$

The standard deviation of the measurements ($n = 9$) using reference gases was ≤0.05 ‰ for both carbon and nitrogen. To compensate for any potential heterogeneity of the analysed material and to achieve reliable results, the average of three analyses was determined for each sample. The standard deviation of these individual analyses was maximally 0.15 ‰, but on average (median) was considerably lower: 0.03 and 0.05 ‰ for carbon in lipids or DDM, respectively, and 0.04 ‰ for nitrogen.

Using the following international standards, sucrose and urea (Merck, Darmstadt) were calibrated as C and N working standards, respectively: IAEA-CH-6 ($\delta^{13}C_{VPDB} = -10.449$ ‰), IAEA-CH-3 ($\delta^{13}C_{VPDB} = -24.724$ ‰) and NBS 22 ($\delta^{13}C_{VPDB} = -30.031$ ‰) for carbon; IAEA-N1 ($\delta^{15}N_{Air} = 0.4$ ‰) and IAEA-N2 ($\delta^{15}N_{Air} = 20.3$ ‰) for nitrogen. These working standards were analysed regularly during each sequence to monitor the repeatability and to calibrate the reference gases (CO_2 and N_2) (Air Liquide, Düsseldorf, Germany).

HPLC determination of carotenoids

The carotenoids in the shrimp tissue were analysed by high-performance liquid chromatography (HPLC) [44]. The investigations were limited to the free astaxanthin fraction.

For the complete extraction of carotenoids, 10 g shrimp homogenate were extracted three times with acetone. The combined extracts of each sample were mixed with water and sodium chloride and then extracted with heptane. The orange-red coloured heptane phase was separated and brought to a definite volume. For the separation of fat, a portion of this sample solution was placed on a small silica gel column and the interfering lipids were flushed from the column. The carotenoids were eluted with methanol. The eluate was concentrated to dryness, and the residue was dissolved in a suitable solvent for HPLC.

This solution was analysed by HPLC in two ways. The levels of free all-*trans*-astaxanthin and canthaxanthin were determined using reversed-phase HPLC, and the separation of the free all-*trans*-astaxanthin isomers was completed using a chiral phase column.

For all samples, duplicate determinations were performed. The mean relative standard deviation of these individual analyses was 5.3 %. The limits of determination for astaxanthin and canthaxanthin were 0.2 µg/g shrimp tissue.

The configurational astaxanthin isomers were identified by comparison of the retention times with those of the synthetic astaxanthin standard and the astaxanthin isolated of the *Phaffia* yeast. The isomers were not base line separated, therefore integration was done by peak height. Synthetic astaxanthin, which was analysed regularly, contained the *(3R,3′R)*, *meso and (3S,3′S)*-form in the ratio 1:2:1.

Partial least-squares discriminant analysis

For the classification of the farmed (conventional, organic) and wild shrimp, a combination of partial least-squares (PLS) and discriminant analysis (DA), the partial least-squares discriminant analysis (PLS-DA), was applied [45, 46]. The PLS is used for the dimension reduction of data sets with many or highly interrelated variables and a small number of objects, without losing too much information for the group affiliation of objects. The PLS analysis extracts successive linear combinations of the predictors, called factors, thereby balancing the two objectives of explaining the response and predictor variations. In the subsequent DA,

the extracted factors from PLS are used as a discriminant criterion function for assortment into predefined classes.

The number of factors (n = 1–8) were accounted for when the predicted residual sum of squares (PRESS) reached a minimum [46, 47]. To avoid "overfitting" and to test the quality of the classification or prediction performance, a cross-validation was conducted. Here, the entire data set was divided in two sets, a training and a test data set. To eliminate any temporal influences, the assignment to the respective groups was completed alternately. The quality of the models was determined by the misclassification rate: the lower the rate, the better the model.

All analyses were performed using the statistical package SAS version 9.2 [45].

Results and discussion

Species identification

The farmed shrimp contained either *P. monodon* or *L. vannamei*. The latter species predominated in organically farmed shrimp. All farmed shrimp were labelled correctly.

Species identification was not possible for all wild shrimp products; in many cases, only the genus could be determined. The wild shrimp products contained animals from the genera *Penaeus*, *Metapenaeus* and *Fenneropenaeus*. These samples were not always homogeneous: some packages apparently contained a mixture of several tropical shrimp species. Packages with heterogeneous content (A-D) are shown in bold in Fig. 3. The results of SSCP analysis, as shown in Fig. 3, demonstrated the polymorphism of ssDNA patterns in the cases of *L. vannamei* and *Fenneropenaeus merguiensis*.

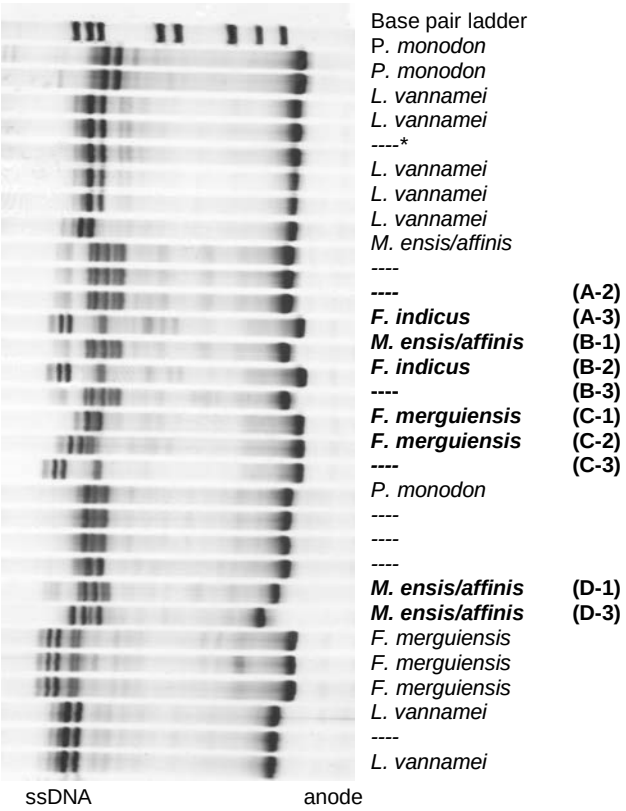

Fig. 3 Differentiation of shrimp by SSCP analysis of a 312 bp sequence of the 16S rRNA gene. CleanGel 10 %, silver staining of DNA bands. Species were identified by sequencing and BLAST analysis. Samples of packages of heterogeneous content (*A–D*) are given in *bold*. *Not sequenced

Proximate composition

The results of the proximate composition of all tropical shrimp analysed are summarised in Table 2. Compared to the wild *Fenneropenaeus* and *Metapenaeus* species, the wild-caught specimens of *P. monodon* contained significantly higher protein levels and lower moisture levels. While the *Fenneropenaeus* and *Metapenaeus* species had an average lipid concentration of 0.6 %, wild *P. monodon* contained approximately 1.1 % lipids.

P. monodon specimens of all production types were available. The low protein and increased moisture contents of conventionally farmed shrimp is substantial when compared with other production types. Many of the conventionally farmed *P. monodon* contained citrate or phosphate, which contribute to higher moisture content. On the other hand, the wild-caught and organically reared *P. monodon* were similar in composition.

The organically farmed *L. vannamei* contained, on average, higher protein and lipid levels than the conventionally produced animals.

Fatty acids

Gas chromatographic lipid analysis included 19 fatty acids. Their relative percentages in the different shrimp species are given in Tables 3 and 4 and are organised according to production method.

In the case of *P. monodon*, the content range within a single type of farming was so wide that characteristic differences of individual fatty acids between conventional and organic aquaculture could not be identified (Table 3). Additionally, a distinction between farmed and wild shrimp was not feasible, due to large fluctuations of the individual fatty acids in the wild animals. However, with one exception, the combination of α-linolenic acid and docosapentaenoic acid allowed the differentiation between organically and conventionally farmed shrimp (Fig. 4) and was indicative of the influence of higher vegetable oil contents in conventional aquaculture feed. A distinction between organic and

Table 2 Proximate composition of tropical shrimp (MV ± SD: mean value ± standard deviation; range: minimum–maximum, based on wet weight; n: number of products)

Species	Production method	*n*	Protein (%) MV ± SD Min–Max	Lipid (%)	Moisture (%)
Individual species per production type					
Fenneropenaeus spp.	Wild	10	12.8 ± 2.6 10.7–18.2	0.6 ± 0.1 0.4–0.8	84.2 ± 2.4 79.1–86.7
Metapenaeus spp.	Wild	4	11.7 ± 0.6 11.2–12.6	0.7 ± 0.1 0.5–0.8	85.4 ± 0.4 85.0–85.7
Metapenaeus & Fenneropenaeus spp.	Wild	4	12.8 ± 1.5 11.1–14.8	0.6 ± 0.1 0.5–0.7	84.7 ± 1.7 82.2–86.2
Penaeus monodon	Wild	7	20.2 ± 2.3 16.9–22.8	1.1 ± 0.2 0.9–1.5	78.3 ± 2.7 75.5–82.2
Penaeus monodon	Organic	11	20.1 ± 0.8 18.8–21.1	1.1 ± 0.4 0.8–2.2	79.3 ± 1.2 77.7–81.1
Penaeus monodon	Conventional	14	15.9 ± 3.4 11.5–23.3	0.9 ± 0.3 0.6–2.0	81.9 ± 3.3 73.8–85.5
Litopenaeus vannamei	Organic	14	17.9 ± 1.7 13.3–20.5	1.3 ± 0.5 0.8–2.8	80.8 ± 1.5 78.8–84.6
Litopenaeus vannamei	Conventional	13	16.5 ± 3.0 13.5–22.4	1.1 ± 0.2 0.9–1.5	81.5 ± 3.4 74.8–85.8
Species pooled per production type					
Fennerop., Metap.spp,, P. monodon	Wild	25	14.7 ± 4.1 10.7–22.8	0.8 ± 0.2 0.4–1.5	82.8 ± 3.6 75.5–86.7
P. monodon, L. vannamei	Organic	25	18.8 ± 1.7 13.3–21.1	1.2 ± 0.4 0.8–2.8	80.1 ± 1.5 77.7–84.6
P. monodon, L. vannamei	Conventional	27	16.2 ± 3.2 11.5–23.3	1.0 ± 0.3 0.6–2.0	81.7 ± 3.3 73.8–85.8

wild *P. monodon* could not be achieved with any combination of fatty acids.

There were clear differences between conventional and organic *L. vannamei* (Table 4). In organically farmed shrimp, the levels of saturated fatty acids, pentadecanoic acid (15:0) and heptadecanoic acid (17:0), were always higher than in conventionally farmed shrimp. Furthermore, the level of the monounsaturated gondoic acid (20:1n9) were often higher in the organically farmed specimens. In contrast, organic *L. vannamei* usually contained lower levels of linoleic acid (18:2n6) and were presumably fed less vegetable oils.

The distribution of fatty acids in wild living *Metapenaeus* and *Fenneropenaeus*, both distributed as *Penaeus* spp., are shown in Table 5. The levels of fatty acids vary widely.

Therefore, fatty acid analysis alone is not suitable for the differentiation between conventional, organic and wild tropical shrimp.

Stable isotopes

Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotopes were analysed. Carbon was recorded in the lipid extract (LIP) and the defatted dry matter (DDM) of the shrimp tissue. Owing to shifted $\delta^{13}C$ content in these two fractions, the fluctuating lipid content within one species resulted in scattering of the $\delta^{13}C$ content in the non-defatted samples. Thus, recording $\delta^{13}C$ content separately in both fractions provides improved interpretation of possible feed influences in different production methods. In addition, the difference between $\delta^{13}C$ content in DDM and LIP was determined for all samples: $\Delta\delta^{13}C = \delta^{13}C_{DDM} - \delta^{13}C_{LIP}$. In the following, the term $\delta^{15}N_{DDM}$ refers to the matrix of the defatted dry matter. Because nitrogen is virtually absent from the extracted lipids, the measured values correspond to the $\delta^{15}N$ of the whole tissue sample.

Table 6 shows the isotopic data of shrimp from different types of production. In the upper part of the table, the results are organised according to a single species. The last three rows of data are summarised according to the three production types.

The combined data of all investigated shrimp are highly variable and did not provide a general distinction between the three production categories: *conventional*, *organic* and *wild*. Further, a differentiation could not be made between organic and conventional aquaculture or between wild and conventionally farmed shrimp. Only wild and organically farmed shrimp could be distinguished from each other using a combination of $\delta^{15}N_{DDM}$ and $\Delta\,\delta^{13}C$ (Fig. 5);

Table 3 Average composition of fatty acids of *P. monodon* from three types of production (% of fatty acids measured; MV ± SD: mean value ± standard deviation; range: minimum–maximum; n: number of products) (SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids)

Fatty acid		Conventional	Organic	Wild
		n = 14	*n* = 11	*n* = 7
Common name	Code	MV ± SD Min–Max		
Myristic acid	14:0	0.73 ± 0.38 0.44–1.92	0.54 ± 0.22 0.43–1.19	0.77 ± 0.30 0.53–1.22
Pentadecanoic acid	15:0	0.80 ± 0.40 0.28–2.02	0.78 ± 0.32 0.46–1.53	1.29 ± 0.53 0.64–2.21
Palmitic acid	16:0	19.14 ± 1.70 17.15–23.10	18.86 ± 1.17 17.18–20.75	17.93 ± 0.91 16.13–18.83
Heptadecanoic acid	17:0	2.43 ± 0.75 1.25–4.55	2.25 ± 0.81 1.62–4.42	3.30 ± 1.49 1.72–5.65
Stearic acid	18:0	12.52 ± 1.38 10.19–14.64	13.61 ± 0.54 12.90–14.56	12.96 ± 0.85 11.31–13.74
∑SFA		35.6	36.0	36.3
Palmitoleic acid	16:1n7	2.29 ± 0.57 1.27–3.32	2.25 ± 0.88 1.46–4.12	4.14 ± 1.94 1.32–6.87
Oleic acid	18:1n9	12.26 ± 1.20 10.53–14.70	13.12 ± 1.41 10.29–14.41	12.53 ± 1.91 10.15–15.32
Vaccenic acid	18:1n7c	3.48 ± 0.54 2.25–4.16	3.00 ± 0.29 2.67–3.67	3.21 ± 0.54 2.23–3.83
Gondoic acid	20:1n9	0.63 ± 0.28 0.25–1.32	0.56 ± 0.17 0.40–0.96	0.88 ± 0.29 0.63–1.26
∑MUFA		18.7	18.9	20.8
Linoleic acid	18:2n6	10.29 ± 2.61 5.16–15.42	11.20 ± 2.40 6.02–13.28	6.92 ± 4.88 2.53–17.37
γ-Linolenic acid	18:3n6	n.d.	n.d.	n.d.
α-Linolenic acid	18:3n3	2.00 ± 0.85 0.77–4.34	1.26 ± 0.43 0.89–2.01	1.51 ± 0.40 1.01–2.15
Stearidonic acid	18:4n3	0.19 ± 0.16 0.05–0.65	n.d.	0.18 ± 0.07 0.07–0.28
Eicosadienoic acid	20:2n6	1.03 ± 0.21 0.67–1.40	1.00 ± 0.09 0.85–1.16	0.95 ± 0.09 0.85–1.10
Arachidonic acid	20:4n6	10.00 ± 2.67 3.63–12.89	10.40 ± 1.30 6.92–11.42	8.74 ± 2.11 5.04–11.10
Eicosapentaenoic acid EPA	20:5n3	11.91 ± 1.23 10.03–14.22	10.48 ± 1.08 9.61–12.68	11.87 ± 0.87 10.55–13.00
Docosatetraenoic acid	22:4n6	n.d.	0.70 ± 0.27 n.d.–0.97	1.08 ± 0.49 0.32–1.63
Docosapentaenoic acid DPA	22:5n3	1.47 ± 0.39 0.71–2.00	1.71 ± 0.20 1.38–2.06	1.89 ± 0.56 0.84–2.54
Docosahexaenoic acid DHA	22:6n3	8.83 ± 1.90 5.26–12.04	**8.31 ± 1.18** 6.73–11.30	9.85 ± 2.06 6.76–12.05
∑PUFA		45.7	45.1	43.0
EPA + DHA		20.7	18.8	21.7

n.d. not detected

Table 4 Average composition of fatty acids of *L. vannamei* of different origin (% of fatty acids measured; MV ± SD: mean value ± standard deviation; range: minimum–maximum; n: number of products) (SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids)

Fatty acid		Conventional	Organic
		n = 13	*n* = 14
Common name	Code	MV ± SD Min–Max	
Myristic acid	14:0	0.59 ± 0.52 0.22–2.22	0.73 ± 0.39 0.44–1.95
Pentadecanoic acid	15:0	0.43 ± 0.09 0.30–0.64	2.55 ± 0.83 1.09–4.53
Palmitic acid	16:0	21.69 ± 1.85 16.67–23.63	20.61 ± 1.53 18.41–24.59
Heptadecanoic acid	17:0	1.56 ± 0.31 0.72–1.82	3.63 ± 0.80 2.49–5.65
Stearic acid	18:0	11.92 ± 1.65 7.16–13.31	11.11 ± 1.37 7.89–12.28
∑SFA		36.2	38.6
Palmitoleic acid	16:1n7	1.50 ± 0.67 1.06–3.63	1.90 ± 0.56 1.30–3.60
Oleic acid	18:1n9c	14.10 ± 3.57 11.17–25.01	13.06 ± 1.79 10.29–17.14
Vaccenic acid	18:1n7c	2.99 ± 0.26 2.64–3.45	2.67 ± 0.53 1.97–3.59
Gondoic acid	20:1n9	0.82 ± 0.36 0.54–1.98	2.00 ± 0.60 0.84–3.23
∑MUFA		19.4	19.6
Linoleic acid	18:2n6c	13.47 ± 1.12 12.20–15.86	10.05 ± 1.40 7.38–13.35
γ-Linolenic acid	18:3n6	n.d.	n.d.
α-Linolenic acid	18:3n3	1.17 ± 0.38 0.80–2.29	1.08 ± 0.26 0.71–1.84
Stearidonic acid	18:4n3	0.20 ± 0.14 0.12–0.53	n.d.
Eicosadienoic acid	20:2n6	1.42 ± 0.20 1.11–1.71	1.19 ± 0.23 0.95–1.77
Arachidonic acid	20:4n6	4.40 ± 0.67 2.62–5.13	5.90 ± 1.17 3.66–7.47
Eicosapentaenoic acid EPA	20:5n3	11.33 ± 2.51 6.37–15.62	10.77 ± 1.13 8.17–12.26
Docosatetraenoic acid	22:4n6	n.d.	0.28 ± 0.18 0.10–0.84
Docosapentaenoic acid DPA	22:5n3	0.95 ± 0.37 0.65–2.11	0.88 ± 0.27 0.54–1.64
Docosahexaenoic acid DHA	22:6n3	11.45 ± 1.44 8.68–13.09	11.59 ± 1.54 9.79–15.37
∑PUFA		44.4	41.7
EPA + DHA		22.8	22.4

n.d. not detected

however, analyses of both the defatted dry matter and the extracted lipids are required. Essentially, these findings indicate a higher ingestion of animal prey by wild compared to conventionally farmed shrimp.

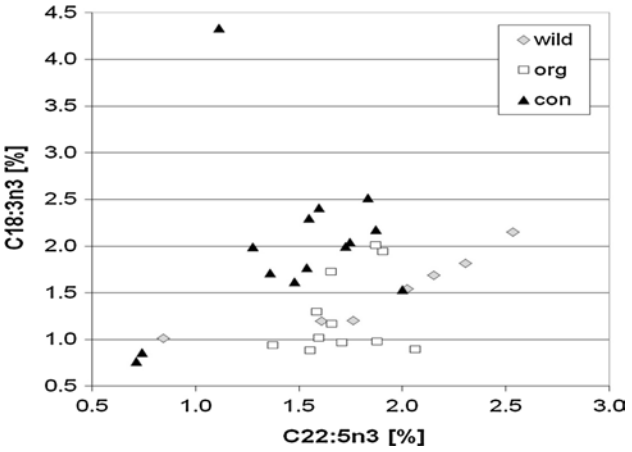

Fig. 4 Docosapentaenoic (22:5n3) and α-linolenic (18:3n3) acid contents (%) of *P. monodon* from three types of production

With respect to individual species, the combination of $\delta^{15}N_{DDM}$ and $\Delta\delta^{13}C$ enabled the distinction of organically and conventionally farmed *L. vannamei* (Fig. 6), whereas this was not possible with *P. monodon*.

Despite the apparently high variability of the nutritional conditions of farmed and wild shrimp a high correlation arose between $\delta^{13}C_{DDM}$ and $\delta^{13}C_{LIP}$ ($r = 0.98$); however, differentiation between growth conditions was still not possible (Fig. 7). This is also reflected in the similar $\Delta\delta^{13}C$ values of the three production types (Table 6).

A differentiation between wild and farmed shrimp was achieved by combining $\delta^{15}N_{DDM}$ with the linoleic acid content (C18:2n6) (Fig. 8). This again shows the infl of vegetable fats in aquaculture feeds, but it does not allow a distinction between organically and conventionally farmed shrimps.

Carotenoids

The carotenoid canthaxanthin was not detected in any shrimp sample. However, all samples contained astaxanthin, from which only the non-esterified, all-*trans* fraction was quantified.

The content and the configurationalisomer distribution of free astaxanthin in the tissue of the 77 tropical shrimp samples examined are summarised in Tables 7 and 8. The levels of free astaxanthin in the shrimp flesh fluctuated both between different *Penaeus* species and within one species.

The percentage distribution of the *meso*-(*3R,3′S*)-form and both enantiomers ((*3R,3′R*) and (*3S,3′S*)) of free astaxanthin in the shrimp meat is shown in Fig. 9. The patterns that were obtained for the organically and conventionally farmed and for the wild-caught shrimps were very similar. *Penaeus* species, production method and country of origin seem to

Table 5 Average composition of fatty acids of different ***wild shrimp*** species (*Metapenaeus* spp. and *Fenneropenaeus* spp). ($n = 18$; % of fatty acids measured; MV ± SD: mean value ± standard deviation; range: minimum–maximum) (SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids)

Fatty acid		*Metapenaeus* spp. + *Fenneropenaeus* spp.
		$n = 18$
Common name	Code	MV ± SD Min–Max
Myristic acid	14:0	1.66 ± 0.57 0.89–3.00
Pentadecanoic acid	15:0	1.12 ± 0.26 0.66–1.63
Palmitic acid	16:0	18.85 ± 0.96 17.01–20.16
Heptadecanoic acid	17:0	2.30 ± 0.54 1.17–3.51
Stearic acid	18:0	11.84 ± 1.98 6.94–15.73
∑SFA		35.8
Palmitoleic acid	16:1n7	6.62 ± 1.33 2.13–8.25
Oleic acid	18:1n9c	12.51 ± 3.32 9.64–21.15
Vaccenic acid	18:1n7c	3.27 ± 0.33 2.27–3.66
Gondoic acid 0.34–2.01	20:1n9	0.70 ± 0.41
∑MUFA		23.1
Linoleic acid	18:2n6c	4.17 ± 2.95 1.56–11.22
γ-Linolenic acid	18:3n6	n.d.
α-Linolenic acid	18:3n3	0.92 ± 0.39 0.53–1.92
Stearidonic acid	18:4n3	0.32 ± 0.19 0.17–0.89
Eicosadienoic acid	20:2n6	0.94 ± 0.12 0.66–1.14
Arachidonic acid	20:4n6	8.90 ± 1.86 4.28–10.61
Eicosapentaenoic acid EPA	20:5n3	12.12 ± 1.70 7.81–14.68
Docosatetraenoic acid	22:4n6	1.08 ± 0.26 0.63–1.52
Docosapentaenoic acid DPA	22:5n3	1.91 ± 0.35 1.43–2.85
Docosahexaenoic acid DHA	22:6n3	10.79 ± 1.06 8.51–12.11
∑PUFA		41.2
EPA + DHA		22.9

n.d. not detected

Table 6 Stable isotope ratios of carbon and nitrogen in different shrimp species from three types of production (MV ± SD: mean value ± standard deviation; Min minimum, Max maximum; n: number of products)

Species	Production	*n*	$\delta^{15}N_{DDM}$ (‰)	$\delta^{13}C_{DDM}$ (‰)	$\delta^{13}C_{LIP}$ (‰)	$\Delta\,\delta^{13}C$ (‰)	
Individual species per production type							
Fennerop. spp.	Wild	10	9.95 ± 1.31	−17.79 ± 1.20	−24.29 ± 0.68	6.50 ± 0.66	MV ± SD
			8.28	−19.94	−25.58	5.39	Min
			11.77	−15.82	−22.96	7.14	Max
Metap. spp.	Wild	4	10.30 ± 0.95	−17.52 ± 0.49	−24.24 ± 0.57	6.72 ± 0.20	MV ± SD
			8.90	−17.99	−24.78	6.43	Min
			10.98	−17.05	−23.59	6.91	Max
Fennerop spp.,	Wild	4	10.69 ± 1.17	−17.01 ± 0.73	−23.75 ± 0.65	6.74 ± 0.25	MV ± SD
Metap. spp.			8.94	−17.80	−24.19	6.38	Min
			11.31	−16.05	−22.79	6.94	Max
P. monodon	Wild	7	7.50 ± 1.23	−20.75 ± 1.98	−26.64 ± 1.64	5.89 ± 0.62	MV ± SD
			5.55	−23.93	−29.45	5.26	Min
			8.89	−18.66	−24.81	7.12	Max
P. monodon	Organic	11	5.09 ± 0.71	−16.62 ± 2.95	−22.71 ± 3.22	6.09 ± 0.31	MV ± SD
			4.44	−23.67	−30.35	5.66	Min
			6.97	−14.74	−20.68	6.68	Max
P. monodon	Conventional	14	5.93 ± 1.44	−19.22 ± 2.49	−25.45 ± 2.49	6.22 ± 0.36	MV ± SD
			4.54	−23.13	−29.06	5.49	Min
			9.39	−15.39	−21.91	6.79	Max
L. vannamei	Organic	14	5.78 ± 0.64	−17.10 ± 1.48	−24.04 ± 1.48	6.94 ± 0.34	MV ± SD
			4.75	−19.93	−26.68	6.34	Min
			6.85	−15.05	−22.41	7.49	Max
L. vannamei	Conventional	13	7.26 ± 0.76	−22.01 ± 0.72	−27.60 ± 0.58	5.59 ± 0.24	MV ± SD
			5.80	−22.78	−28.22	5.15	Min
			8.76	−20.66	−26.56	6.09	Max
Species pooled per production type							
Fennerop. spp.,	Wild	25	9.44 ± 1.70	−18.45 ± 1.96	−24.86 ± 1.51	6.40 ± 0.62	MV ± SD
Metap. spp.			5.55	−23.93	−29.45	5.26	Min
P. monodon			11.77	−15.82	−22.79	7.14	Max
P. monodon.	Organic	25	5.48 ± 0.74	−16.89 ± 2.21	−23.45 ± 2.44	6.56 ± 0.54	MV ± SD
L. vannamei			4.44	−23.67	−30.35	5.66	Min
			6.97	−14.74	−20.68	7.49	Max
P. monodon.	Conventional	27	6.57 ± 1.33	−20.56 ± 2.31	−26.48 ± 2.11	5.92 ± 0.44	MV ± SD
L. vannamei			4.54	−23.13	−29.06	5.15	Min
			9.39	−15.39	−21.91	6.79	Max

be irrelevant for the configurational isomer distribution. The average percentage ratio of *(3R,3′R)*: *meso: (3S,3′S)* in all samples was 17:38:45. Because the wild and farmed shrimp consumed different diets, it is likely that metabolism and racemisation of the consumed carotenoids occurred [29, 36].

Schiedt et al. [36] were able to demonstrate that *Penaeus japonicus* is capable of converting optically pure (3S,3′S)-astaxanthin into the meso- and (3R,3′R)-isomers, and the tissue contained the (3R,3′R): *meso:* (3S,3′S)-forms of free astaxanthin in the ratio of 13:43:44. When analysing esterifi astaxanthin of these samples, the isomers were present in the approximate ratio of 1:2:1, the ratio was thus shifted in favour of the meso form.

In a study with wild-caught *Penaeus* species, Latscha [30] showed that *L. vannamei* and *P. monodon* contained the (3R,3′R): *meso:* (3S,3′S)-isomers in the ratio of 23:44:32 and 19:45:36, respectively. The results of our study are in accordance with this distribution.

Partial least-squares discriminant analysis (PLS-DA)

The described methods were not able to distinguish between the three shrimp origins—wild, organic and

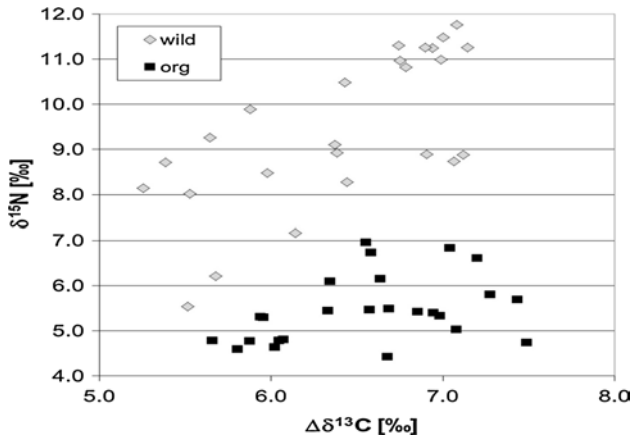

Fig. 5 $\Delta\delta^{13}C$ and $\delta^{15}N_{DDM}$ of different shrimp species from various origins

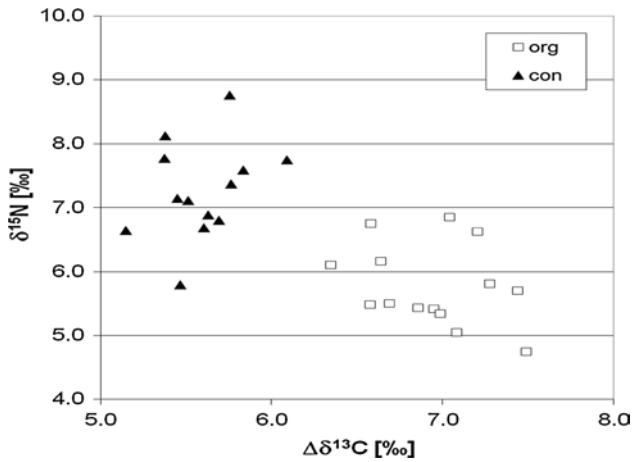

Fig. 6 $\Delta\delta^{13}C$ and $\delta^{15}N_{DDM}$ of differently farmed *L. vannamei*

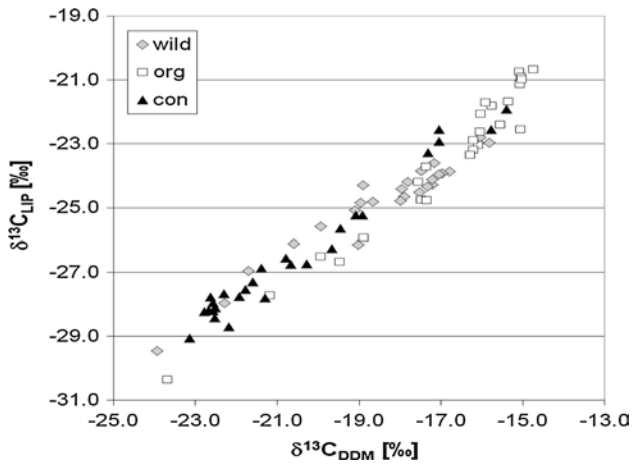

Fig. 7 $\delta^{13}C_{DDM}$ and $\delta^{13}C_{LIP}$ of different shrimp species from various types of production

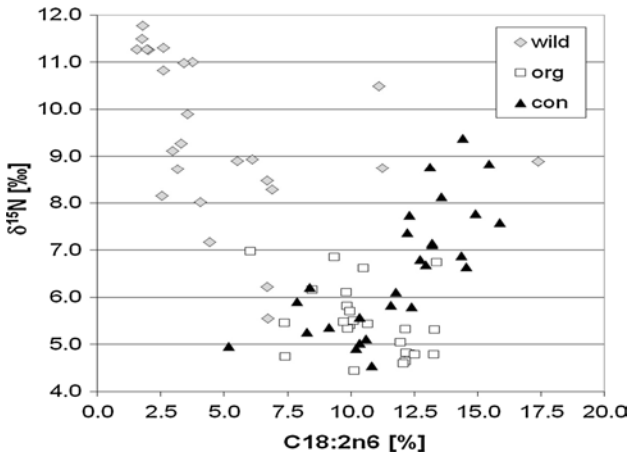

Fig. 8 $\delta^{15}N_{DDM}$ and C18:2 of different shrimp species from various types of production

conventional—without considering the shrimp species. For this reason, all possible methodological combinations were tested using PLS-DA.

Analysis of the total fatty acid data allowed an 87 % correct classification (Table 9). Although mismatches occurred in each of the three groups, there were no false positive assignments of conventionally farmed or wild shrimp as organically farmed animals.

The inclusion of stable isotopes to the fatty acid data did not improve the hit rate of 87 %, but led to the false positive classification of one conventionally farmed sample as organic. Despite the higher analytical effort, this model did not offer any improvement, and it was concluded that the two analytical methods provide similar information. Though, as described above, the stable isotope data does contain some significance.

The combination of fatty acid and carotenoid (astaxanthin content and isomer pattern) data allowed a 92 % correct assignment with 100 % detection of organically farmed shrimp (Table 10). Only the distinction of conventionally farmed and wild shrimp was incomplete. Hence, a check for a correct organic labelling would be possible.

The use of stable isotopes and carotenoids for the PLS-DA also allowed, at a slightly lower rate of 87 % correct assignments, a correct classification of all organically farmed shrimp, but a part of the conventionally farmed shrimp was assigned to the organic group as well. Therefore, the combination of stable isotope and carotenoid analysis was less suitable for authenticating organic shrimp than that of fatty acid and carotenoid analysis.

The PLS-DA of stable isotope, fatty acid and carotenoid data did not improve the results and is comprised of unrealistically high analytical efforts for use in daily practice.

Table 7 Content of free all-*trans* astaxanthin and percentage ratios of the configurational isomers in shrimp tissue of different species (MV ± SD: mean value ± standard deviation; Min: minimum; Max: maximum; n: number of products)

Production method	*Litopenaeus vannamei*			*Penaeus monodon*			*Metapenaeus* spp. & *Fenneropenaeus* spp.		
	Organic & conventional			Organic & conventional & wild			Wild		
n	27			32			18		
	Min	MV ± SD	Max	Min	MV ± SD	Max	Min	MV ± SD	Max
Astaxanthin (µg/g)									
All-*trans* astaxanthin	0.53	1.34 ± 0.54	3.00	0.40	1.80 ± 0.95	3.75	0.18	0.47 ± 0.33	1.35
Astaxanthin isomers (%)									
3R,3′R-isomer	15.2	16.4 ± 0.9	18.8	12.6	15.4 ± 1.9	21.3	16.1	21.1 ± 3.9	31.7
3R,3′S-isomer	35.4	36.8 ± 0.9	38.7	33.8	37.9 ± 1.3	40.8	34.9	39.2 ± 2.2	43.5
3S,3′S-isomer	43.2	46.8 ± 1.3	48.9	38.3	46.7 ± 2.8	52.0	29.6	39.6 ± 4.5	46.3

Table 8 Content and distribution of configurational isomers of free all-*trans* astaxanthin in the tissue of *L. vannamei* and *P. monodon* from three production types (MV ± SD: mean value ± standard deviation; range: minimum–maximum; n: number of products)

Production method	*Litopenaeus vannamei*		*Penaeus monodon*		
	Organic	Conventional	Organic	Conventional	Wild
n	14	13	11	14	7
All-*trans* astaxanthin					
MV ± SD (µg/g)	1.22 ± 0.39	1.47 ± 0.66	2.60 ± 0.91	1.41 ± 0.75	1.34 ± 0.50
Min–Max (µg/g)	0.53–1.75	0.58–3.00	1.43–3.75	0.40–3.53	0.73–1.88
Astaxanthin isomers					
3R,3′R-isomer (%)					
MV ± SD	16.1 ± 0.9	16.7 ± 0.9	15.2 ± 0.9	14.4 ± 1.2	17.7 ± 2.6
Min–Max	15.2–18.8	15.5–18.1	13.8–16.3	12.6–16.9	15.0–21.3
3R,3′S-isomer (%)					
MV ± SD	36.3 ± 0.7	37.3 ± 0.8	38.2 ± 0.6	37.3 ± 1.5	38.8 ± 1.3
Min–Max	35.4–37.2	35.7–38.7	37.4–39.3	33.8–40.6	36.5–40.8
3S,3′S-isomer (%)					
MV ± SD	47.6 ± 1.1	45.9 ± 1.0	46.6 ± 1.3	48.2 ± 2.0	43.6 ± 3.5
Min–Max	45.7–48.9	43.2–47.0	44.7–48.7	44.7–52.0	38.3–48.6

Considering the species-specific data, as described above, specimens of *L. vannamei* could be differentiated into organically and conventionally farmed groups using stable isotopes (Fig. 6) or fatty acids. Wild *L. vannamei*, however, were not included in the analysis.

The combination of two fatty acids allowed a largely distinction between organically and conventionally farmed *P. monodon* (Fig. 4), but not between farmed and wild samples. Considering the production types, the PLS-DA of all fatty acids resulted in a 60 % hit rate, with 2/3 of the organically and 1/3 of the conventionally farmed shrimp being correctly assigned. However, if only the farmed animals were used in the model (Table 11), a total of 92 % of the samples, 83 % of organically and 100 % of conventionally farmed samples, were correctly assigned. When any of the three possible paired combinations of stable isotopes, fatty acids and carotenoids were used, an improvement in the total number of correctly assigned samples was not observed. Only the PLS-DA of data from all three analytical methods yielded 100 % correct classification or distinction between organically and conventionally farmed *P. monodon* (Table 12).

With the inclusion of data from wild grown *P. monodon*, a complete differentiation between the three origins could not be achieved using multidimensional data of one method ($n > 2$) nor by a combination of two or even three analytical methods. The species-specific PLS-DA of *P. monodon* only provided distinct results with respect to the authentication of organic products by neglecting the wild shrimp and combining the three methods of analysis. In contrast, multivariate modelling was not required for *L. vannamei*. Although a positive result (1 incorrect assignment) was achieved for *P. monodon* using a combination of docosapentaenoic and α-linolenic acid data (Fig. 4).

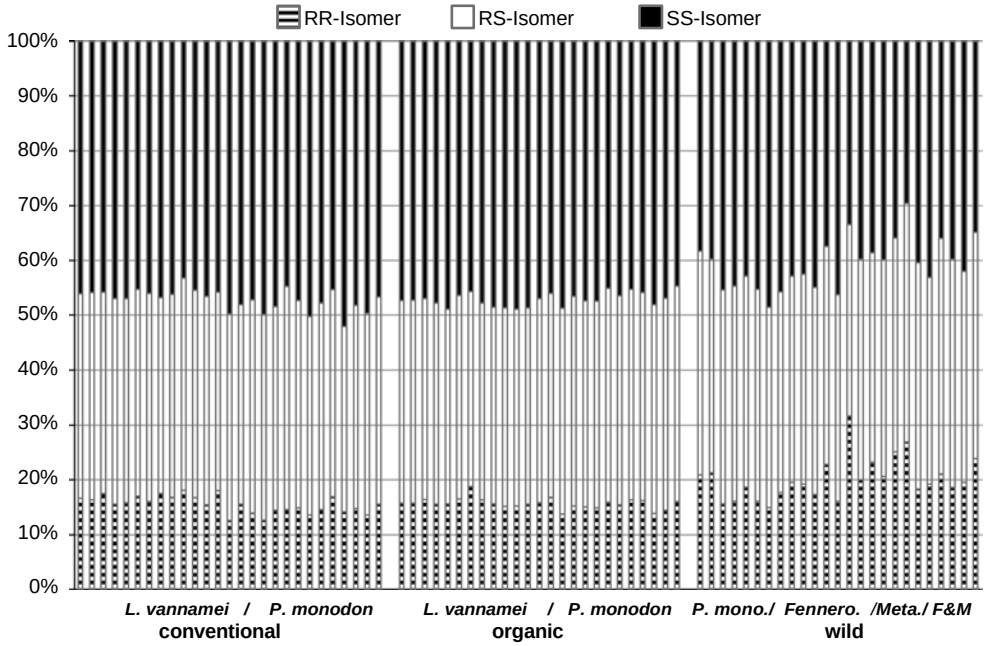

Fig. 9 Percentage distribution of configurational isomers of free all-*trans* astaxanthin [(*3R,3′R) = RR; (3R,3′S) = RS;* (*3S,3′S*) = SS] in different wild and farmed shrimp species

Table 9 Classification of shrimp of different species and types of production by PLS-DA; factors: fatty acids (n = 2 factors, correct classification total = 87 %)

Actual group production	Predicted group production		
	Organic	Conventional	Wild
Organic	85 %	15 %	–
Conventional	–	92 %	8 %
Wild	–	17 %	83 %

Table 10 Classification of shrimp of different species and types of production by PLS-DA; factors: fatty acids and carotenoids (n = 4 factors, correct classification total = 92 %)

Actual group production	Predicted group production		
	Organic	Conventional	Wild
Organic	100 %	–	–
Conventional	–	85 %	15 %
Wild	–	8 %	92 %

Table 11 Classification of farmed *P. monodon* to the type of production by PLS-DA; factors: fatty acids (n = 1 factor, correct classification total = 92 %)

Actual group production	Predicted group production	
	Organic	Conventional
Organic	83 %	17 %
Conventional	–	100 %

Table 12 Classification of farmed *P. monodon* to the type of production by PLS-DA; factors: stable isotopes, fatty acids and carotenoids (n = 1 factor, correct classification total = 100 %)

Actual group production	Predicted group production	
	Organic	Conventional
Organic	100 %	–
Conventional	–	100 %

Conclusion

The stable isotope ratios of carbon and nitrogen in all shrimp samples were variable. Only a distinction between wild and organically farmed shrimps was apparent using a combination of $\delta^{15}N_{DDM}$ and $\Delta\delta^{13}C$. A substantial, yet incomplete, distinction of wild and farmed shrimps was achieved by combining $\delta^{15}N_{DDM}$ with linoleic acid (C18:2n6) levels. A differentiation between organic and conventional aquaculture was achieved for *L. vannamei* using $\delta^{15}N_{DDM}$ and $\Delta\delta^{13}C$.

Organically farmed specimens of *P. monodon* were distinguished from conventionally farmed specimens using a combination of α-linolenic acid and docosapentaenoic acid. However, the high variation of fatty acid contents in wild shrimp prevented their distinction from farmed shrimp

by this means. Farmed *L. vannamei* were assigned to an organic or conventional origin on the basis of pentadecanoic acid or heptadecanoic acid.

The carotenoid analyses resulted in a uniform distribution of the free astaxanthin configurational isomers irrespective of the shrimp species, the country of origin and the mode of production. Thus, a distinction between wild, conventionally and organically farmed shrimps by their astaxanthin isomer distribution is not possible.

A differentiation between organically and conventionally farmed and wild living Penaeidae was not possible with the analysis of stable isotopes, fatty acids or carotenoids in the shrimp tissue.

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