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Traceability of organic fish – authenticating the production origin of salmonids by chemical and isotopic analyses

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Abstract

To develop a practical methodology for the authentication of organic salmonid products, 130 fillet samples of trout and salmon originating from organic and conventional aquaculture as well as wild stocks (salmon) were collected from the German market over one year. Combined stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$ in defatted dry matter allowed differentiation of organically farmed from conventionally farmed salmon and brown trout, whether raw, smoked or graved. For the additional distinction of organic and wild salmon, a second analysis of $\delta^{13}C$ in fish lipids was required. Fatty acid analysis completely differentiated the three production types of salmon just by the linoleic acid content in the fish lipids, which was lowest in wild and highest in conventional salmon. Moreover, the elevated myristic acid content allowed organic to be distinguished from wild and conventional salmon. Furthermore, organic and conventional brown trout could be distinguished by combining the oleic acid and gondoic acid contents. Analysis of the free astaxanthin isomeric pattern allowed a clear distinction of conventional and wild salmon, but organic salmon showed variable patterns that did not consistently allow the authentication of their origin. While a special feed composition is required in organic aquaculture, the composition of conventional aquaculture feed has changed considerably within the last decade. Consequently, the percentages of animal and vegetable components, which clearly vary between the production types, result in distinctive features in terms of stable isotope or fatty acid composition that are utilisable for the authentication of organic salmonid products. To account for potential changes in aquaculture feeding practices, the established distinctive limits should be traced and possibly adapted in future.

Keywords

Organic fish; Salmonids; Authentication; Stable isotopes; Fatty acids; Carotenoids

1. Introduction

The dwindling natural resource of wild fish has led to an increasing importance of aquaculture for the production of edible fish. In recent decades, organic fish production has begun to compete with traditional aquaculture. Hence, appropriate instruments for the traceability of fish products are needed with respect, in particular, to European regulation (EC) 710/2009 on organic aquaculture animal production, which amended the EU rules on organic farming (EC, 2009). To counteract the potential risk for conventional products being wrongly labelled as organic, appropriate laboratory methods that are applicable on the trading level are of great interest.

Several studies have already investigated the differentiation between wild and cultured fish. With salmonids, compositional as well as isotopic parameters were applied, including differentiators derived from analyses of fatty acids (Ackman & Takeuchi, 1986; Axelson, Standal, Martinez, & Aursand, 2009; Blanchet, et al., 2005; Hamilton, et al., 2005; van Vliet & Katan, 1990), carotenoids (Lura & Sægrov, 1991; Turujman, Wamer, Wei, & Albert, 1997), stable isotopes (Anderson, et al., 2010; Dempson & Power, 2004; Schröder & de Leaniz, 2011) and ^{13}C-NMR patterns (Aursand, et al., 2009) as well as elemental analysis (Anderson, Hobbie, & Smith, 2010) or combinations of the aforementioned differentiators (Aursand, Mabon, & Martin, 2000; Martinez, Standal, Axelson, Finstad, & Aursand, 2009; Thomas, et al., 2008). However, the distinction between fish from organic and conventional aquaculture is less thoroughly investigated.

The first results on the authentication of organic fish were published by the authors of the present study and addressed the production of Atlantic salmon (*Salmo salar*). The analysis of carotenoids could not always unambiguously identify organic salmon (Ostermeyer & Schmidt, 2004), whereas the combination of the stable isotope ratio of nitrogen (δ^{15}N) from fish muscle with the linoleic acid content (18:2n6) of fish lipids allowed distinction between wild, organically and conventionally farmed salmon (Molkentin, Meisel, Lehmann, & Rehbein, 2007). Both studies are based on the recognised fact that the composition of fish meat is influenced by the nature of the food ingested, as described in numerous papers.

In conventionally working aquaculture farms, the feed ingredients of animal origin such as fish meal or fish oil are increasingly being replaced by vegetable products such as cereals and vegetable oils for economic reasons. However, for carnivorous species such as salmon or trout, the EU guidelines for organic farming

require a minimum of 40% animal content (EC, 2009), which must originate from organic production or be processing residuals from sustainable fisheries. In contrast, more than 60% vegetable ingredients are permissible in conventional aquaculture feed for carnivores. Hence, the variable contents of animal and vegetable proteins or lipids may offer potential for distinguishing organic and conventional as well as wild salmonids by fatty acid or stable isotope analysis.

The stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are particularly informative with respect to certain food components. The variation in $\delta^{13}C$ largely results from the different mechanisms of CO_2 fixation between C3 and C4 plants, with C4 plants such as maize having higher $\delta^{13}C$. Moreover, $\delta^{13}C$ and, in particular, $\delta^{15}N$ continuously increase along the food chain (Minagawa & Wada, 1984). Hence, animal protein typically shows higher $\delta^{15}N$ than vegetable protein. Because the isotope ratios in fish tissue reflect their previous diet (Moreno-Rojas, Tulli, Messina, & Guillou, 2008), differing food sources might help to distinguish the fish from different production types.

Similarly, the fatty acid composition of fish lipids is significantly influenced by their dietary sources, which was previously described for Atlantic salmon (*S. salar*) (Hamilton, et al., 2005). Because plant oils are often rich in oleic or linoleic acid, these fatty acids will be present in the fish lipids depending on the percentage of plant lipids in the feed. Hence, the differing plant oil contents in the food sources of organic aquaculture compared to conventional aquaculture or wild grown fish are a potential differentiator of production types.

The characteristic red colour of salmon flesh is due to carotenoids, which the animals ingest with their diet. Astaxanthin is the main carotenoid found in the flesh of wild salmon. It is produced in the marine habitat by microalgae and phytoplankton, which are eaten by zooplankton and crustaceans. The crustaceans are subsequently eaten by fish, so that astaxanthin accumulates in the food chain (Yuan, Peng, Yin, & Wang, 2011). Canthaxanthin can only be found in relatively low concentrations, if at all (Putnam, 1991; Shahidi, Metusalach, & Brown, 1998). The consumer typically expects that the flesh of farmed salmon is similarly coloured, so pigments must be added to their feed (Whyte, Travers & Sherry, 1998).

According to European regulation (EC) 1831/2003 (EC, 2003), salmon as well as trout may be reared with feed containing astaxanthin and/or canthaxanthin. For economic reasons, synthetic dyes are usually added in conventional aquaculture, in

particular for salmon. In European organic regulation (EC) 889/2008 (EC, 2008), the use of astaxanthin for food from carnivorous aquaculture animals is regulated. Astaxanthin derived primarily from organic sources, such as the shells of organic crustaceans, may be used in the diet for salmon and trout. If organic sources are not available, natural sources of astaxanthin, such as Phaffia yeast, may be used. Synthetic pigments are not permitted.

Because our previous study exclusively investigated raw salmon fillets (Molkentin, et al., 2007), the aim of the present study was to examine the applicability of organic salmon indicators to processed products while concurrently checking the actual validity of the previously established parameters. In the present study, wild salmon were represented by *Oncorhynchus nerka* due to their higher market share compared to wild *Salmo salar*. Moreover, the current project included raw and processed samples of brown trout (*Salmo trutta*) to examine the potential of authenticating additional organically produced salmonids. Hence, stable isotope, fatty acid and carotenoid analyses were performed on products from three salmonid species to distinguish their different production origins with a focus on the different aquaculture forms.

2. Materials and Methods

2.1. Samples

Smoked and graved salmon products (n = 58) were purchased between November 2010 and January 2012 from retail stores or at wholesale in Hamburg, Germany. The organically and conventionally farmed salmon belonged to the Atlantic salmon (*Salmo salar*) species and originated from aquaculture in Ireland, Scotland and Norway. The wild salmon, which was caught near Alaska, almost exclusively comprised Pacific salmon (*Oncorhynchus nerka*), except for one sample (*Oncorhynchus kisutch*). Unprocessed (n = 55) and smoked (n = 17) samples of brown trout (*Salmo trutta*) were repeatedly purchased in the autumn of 2010 and 2011 directly from several conventional and organic fish farms located in Germany. More detailed information on the 130 samples is given in **Table 1**.

To obtain a homogeneous sample of sufficient quantity, several fish or processed products from the same batch were pooled if necessary. After cutting the fillet material and taking aliquots for carotenoid and species analysis, the samples

were further homogenised using an Ultra-Turrax dispersing apparatus (IKA, Staufen, Germany), and all subsamples were stored at -40 ℃ until processing.

Fish lipids were extracted from 12.5 g of fish homogenate based on the method of Smedes (1999) using cyclohexane and 2-propanol and then determined quantitatively. Along with the sediment, the remaining aqueous layer was lyophilised to obtain the defatted dry matter (DDM). The isolated components were stored at -20 ℃ until analysis.

Samples of aquaculture feed were obtained from 3 conventional (A, B, C) and 3 organic (D, E, F) trout farms in Germany in the autumn of 2010 and 2011, respectively. The feed lipids and the DDM were prepared as described for fish. The stable isotope analyses comprised only samples from 2010, whereas the fatty acid results included samples from both years and were averaged per farm.

2.2. Species identification

2.2.1 Isoelectric focusing (IEF) of water-soluble proteins

The species identification was performed according to (BVL, 2002). A 5 g portion of light muscle was extracted by 15 mL of distilled water. The insoluble protein was removed by centrifugation. The water-soluble proteins were separated using Servalyt Precotes® 3-10 polyacrylamide gels (SERVA, Heidelberg, Germany) and subsequently stained using SERVA Violet 17 Coomassie dye (SERVA). IEF Marker 3-10 (Liquid Mix; SERVA, Cat. No. 39212) was used as the pl marker.

2.2.2 *PCR based DNA analys*is

A segment of the mitochondrial $tRNA^{Glu}$/Cytochrome b genes comprising 464 base pairs was amplified and subsequently sequenced (Schiefenhövel & Rehbein, 2011). The obtained sequences were compared within the sample set and to the sequences in GenBank (http://www.ncbi.nlm.nih.gov/) using **b**asic **l**ocal **a**lignment **s**earch **t**ool (BLAST). Furthermore, the PCR-single strand conformation polymorphism (SSCP) method was applied to the 464 bp amplicon to test the uniformity of the salmon and trout batches (Rehbein, 2005).

2.3. Stable isotopes

2.3.1 Sample preparation

Samples of the lipids (460 µg) and the DDM (750 µg), which were prepared as described in section 2.1, were weighed into tin capsules and combusted using a Thermo Scientific Flash EA 1112 elemental analyser (Waltham, MA) as described previously (Molkentin & Giesemann, 2007). The separated reaction gases were subjected to online MS analysis.

2.3.2 Stable isotope analysis and calibration

The lipid samples (LIP) were analysed for carbon and the DDM samples were simultaneously examined for both carbon and nitrogen stable isotopes using a Deltaplus XL isotope-ratio mass spectrometer with Isodat 1.5 software (Thermo Scientific). Isotope ratios of $^{13}C/^{12}C$ (and the corresponding $^{15}N/^{14}N$ ratios) were expressed in ‰ on a δ-scale as follows:

$$\delta^{13}C\ [‰] = [\ (R_{sample} / R_{standard}) - 1\ ] \times 1000; \qquad R = {}^{13}C / {}^{12}C$$

The δ-values refer to the international VPDB standard for carbon and the AIR standard for nitrogen. The following international secondary standards were used to calibrate the laboratory working standards of urea and sucrose (Merck, Darmstadt, Germany): IAEA-CH-3 ($\delta^{13}C_{VPDB}$ = -24.724‰), IAEA-CH-6 ($\delta^{13}C_{VPDB}$ = -10.449‰), and NBS 22 ($\delta^{13}C_{VPDB}$ = -30.031‰) for carbon; and IAEA-N1 ($\delta^{15}N_{Air}$ = 0.4‰) and IAEA-N2 ($\delta^{15}N_{Air}$ = 20.3‰) for nitrogen. To monitor the measurement repeatability and to calibrate the nitrogen and carbon dioxide reference gases (Air Liquide, Düsseldorf, Germany), the working standards were analysed regularly during each sequence. The standard deviation of the reference gas analyses (n = 9) was ≤ 0.05‰ for both carbon and nitrogen. All samples were analysed in triplicate to compensate for any heterogeneity and the results were expressed as the mean. The standard deviation of the triplicates was always ≤ 0.15‰, with median values of 0.03‰ for $\delta^{13}C$ in LIP, 0.05‰ for $\delta^{13}C$ in DDM and 0.04‰ for $\delta^{15}N$ in DDM. In short, the following parameters were determined for each sample: $\delta^{13}C_{LIP}$, $\delta^{13}C_{DDM}$, $\delta^{15}N_{DDM}$ and, for trout samples only, the difference $\Delta\delta^{13}C$ (= $\delta^{13}C_{DDM}$ - $\delta^{13}C_{LIP}$) as well as the sum $\Sigma\delta_{DDM}$ (= $\delta^{15}N_{DDM}$ + $\delta^{13}C_{DDM}$).

224 *2.4. Fatty acids*

225 Fatty acid methyl esters (FAMEs) were prepared from the extracted lipids,
226 which were dissolved in cyclohexane, by trans-esterification of the triacylglycerols
227 using potassium hydroxide (DGF, 1998). The resulting FAME solution was diluted
228 1:20 and 1 µL was analysed according to (DGF, 2000) on a GC 7890A gas
229 chromatograph (Agilent, Santa Clara, CA) equipped with a flame ionisation detector,
230 a splitless injection port and a 60 m x 0.32 mm J&W DB-23 column with 0.25 µm film
231 thickness (Agilent). The helium carrier gas flow was a constant 1.0 mL min^{-1}, the
232 injector temperature was 250 °C and the detector temperature 300 °C. The
233 temperature program was as follows: start at 140 °C for a 5 min isothermal hold, then
234 2 °C min^{-1} up to 160 °C with a 10 min isothermal step, followed by 1 °C min^{-1} up to
235 180 °C, then a 5 min isothermal hold, 2 °C min^{-1} up to 250 °C, and finally a 5 min
236 isothermal hold. Evaluation with the ChemStation software (Agilent) included 19
237 individual fatty acids in the range of C14:0 – C22:6 and their sum was set to 100%.
238 Hence, the fatty acid contents are given in per cent of measured fatty acids (g/100 g).
239
240 *2.5. HPLC determination of carotenoids*

241 The carotenoids in the tissue of salmon and brown trout were analysed by
242 HPLC (Ostermeyer & Schmidt, 2004). For the complete extraction of carotenoids, 10
243 g of fish homogenate were extracted thrice with acetone. The combined extracts of
244 each sample were mixed with water and sodium chloride and then extracted with
245 heptane. The visibly coloured heptane phase was separated and filled up to a
246 definite volume. For the separation of lipids, a portion of this sample solution was
247 cleaned by solid phase extraction on a small silica gel column. The lipids were
248 flushed from the column, and the carotenoids were subsequently eluted with
249 methanol. The eluate was concentrated to dryness and the residue was taken up in a
250 suitable solvent for HPLC. The all-*trans*-astaxanthin and canthaxanthin contents were
251 determined with reversed-phase HPLC. For the separation of the configurational
252 astaxanthin isomers, a chiral stationary phase was used.

253 Duplicate determinations were performed in all samples. The mean relative
254 standard deviation of these single quantitative analyses was 2.8%. The calibration
255 curves were linear between 0.02 and 2.0 µg/mL.

256 The configurational astaxanthin isomers were identified by comparison of their
257 retention times with those from the synthetic astaxanthin standard and the

astaxanthin isolated from the yeast *Phaffia rhodozyma.* The synthetic astaxanthin contained (*3R,3'R*), meso and (*3S,3'S*) forms in a ratio of 1:2:1, whereas the (*3R,3'R*)-isomer was the only form present in the yeast isolate. Because the isomers were not base line separated, integration was conducted by peak height.

2.6. Statistical analysis

SigmaPlot 11.0 (Systat Software, Inc., San José, CA) was used to perform a statistical analysis of selected data on a 5% level of significance ($P < 0.05$). Depending on the normality test of raw data, Student's t-test or Mann-Whitney's test were used for a pairwise determination of differences between group mean values. Because for a complete distinction of individuals from different production types any overlap of data ranges must be avoided, a comprehensive comparison of mean values of the analysed parameters was not meaningful with respect to the aim of this study.

3. Results and Discussion

3.1. Species identification

To investigate the applicability of analytical methods for the authentication of organically produced salmonids, samples from different production types and origins of salmon (n = 58) and trout (n = 72) were examined (**Table 1**). Before compositional analysis, the declared species of each sample was verified by protein and DNA analysis.

The salmon samples were first analysed by SSCP to check the uniformity of the batches. To confirm these results, DNA sequencing (BLAST) was performed on a selection of 23 samples resulting in a compliance of ≥99%. All salmon samples were labelled correctly. The farmed salmon belonged to the species *S. salar* and the wild salmon comprised the species *O. nerka* and *O. kisutch*. Because of endangerment of wild fish stocks, wild *S. salar* is rarely traded today. Hence, we aimed at analytically distinguishing the three production types, *organic*, *conventional* and *wild*, using typical marketed products, even though they belonged to different species. This allows the use of a single easy-to-use method in the daily practice of food control, although fish of a different species could also be identified by the methods above. However, the primary goal was the authentication of organic products.

The raw trout samples were analysed by IEF first, whereas the smoked trout samples were subjected to SSCP analysis because certain proteins are denatured by the smoking process. Selected DNA sequencing confirmed that all samples belonged to the species *Salmo trutta* (brown trout).

3.2. Stable isotopes

3.2.1. Salmon

Although nitrogen stable isotopes were analysed in the DDM, the obtained values practically match the $\delta^{15}N$ of the whole fish tissue homogenate, as the LIP fraction is almost free of nitrogen. However, $\delta^{13}C$ was recorded separately in LIP and DDM to eliminate the scattering total $\delta^{13}C$ values due to the $\delta^{13}C$ shift between both fractions as well as the fluctuating lipid content within the individual fish in one batch, origin or species.

In our previous study on organic salmon identification (Molkentin, et al., 2007), we did not succeed in differentiating organically from conventionally farmed fish using only $\delta^{15}N$ and $\delta^{13}C$. This was mainly due to the high variation of both parameters in salmon from conventional aquaculture. However, we only recorded the total $\delta^{13}C$ of the fish tissue in that study. Consequently, at that time, the authentication of organic salmon required the combination of $\delta^{15}N$ with the linoleic acid content in the lipid fraction.

Table 2 shows the isotopic data of the salmon recorded in the present study. Because the type of processing did not cause significant differences, graved and smoked products were combined and the data subdivided only into wild, organic and conventional production. None of the listed parameters alone was suitable to differentiate all three production types, as their ranges overlapped at least between two categories. On average, $\delta^{15}N_{DDM}$ was higher in organic and wild salmon compared with conventional. Because the same applies to $\delta^{13}C_{DDM}$, a good correlation ($r = 0.90$) between both parameters resulted (**Fig. 1**). However, organic salmon could not be distinguished from wild using only DDM parameters, although both groups were clearly different from conventional salmon. The slightly elevated DDM values of organic vs. wild salmon probably were caused by the ingestion of fish meal originating from a higher food chain level than wild salmon's prey (Minagawa & Wada, 1984; Molkentin, et al., 2007). The cause of the overall decreased δ-values in conventional salmon was most likely from the higher percentage of vegetable feed

ingested (Torstensen, et al., 2008). In contrast to our previous study (Molkentin, et al., 2007), apparently all conventional aquaculture farms currently use low percentages of animal feed components, leading to a consistently low $\delta^{15}N_{DDM}$ level.

For $\delta^{13}C_{LIP}$, the organic salmon on average showed higher values than conventional and wild salmon (**Table 2**), whereas wild and conventional salmon had similarly lower values due to different reasons. However, the combination of $\delta^{15}N_{DDM}$ and $\delta^{13}C_{LIP}$ allowed complete separation of the three production types (**Fig. 2**). While conventional salmon could be distinguished from wild and organic by $\delta^{15}N_{DDM}$, organic salmon differed from wild and conventional by its $\delta^{13}C_{LIP}$ value. Although $\delta^{13}C_{DDM}$ did not allow differentiation of organic from wild salmon, $\delta^{13}C_{LIP}$ showed distinctly lower values in wild salmon. The cause for the higher depletion of ^{13}C in wild salmon lipids may be an increased proportion of fatty acids originating from endogenous lipogenesis (DeNiro & Epstein, 1977) compared to salmon from organic aquaculture, which obtain a high fat intake. On the other hand, the low $\delta^{13}C_{LIP}$ in conventional salmon is caused, despite a high fat intake, by the decreased ingestion of animal derived feed. Hence, separately recording $\delta^{13}C$ in both the DDM and LIP fractions provided improved information on feed influences in different production types.

As **Fig. 2** shows, the distinction between organic and conventional aquaculture can already be made by either $\delta^{15}N_{DDM}$ or $\delta^{13}C_{LIP}$. However, one sample of smoked salmon declared as organic, which is not shown in **Figs. 1** and **2**, produced results of $\delta^{15}N_{DDM}$ = 10.0‰ and $\delta^{13}C_{LIP}$ = -27.6‰, which are typical of conventional salmon. According also to the analysis of fatty acids (section 3.3.1.) and carotenoids (section 3.4.1.), this sample was clearly assigned to a conventional production origin; therefore, an erroneous or fraudulent declaration must be supposed. We interpreted this finding as confirmation rather than as contradiction of our distinctive features.

According to **Fig. 2**, the differentiation of the three production types was independent of the type of processing, i.e., graved and smoked salmon products produced similar results, although smoked salmon showed somewhat greater variation. As mentioned above, salmon from organic and conventional aquaculture belonged to the species *S. salar* (Atlantic salmon), whereas wild salmon comprised Pacific salmon with samples of *O. nerka* and one of *O. kisutch*. While the authentication parameters were intentionally established across different species due to practical reasons, the average $\delta^{15}N_{DDM}$ for Pacific salmon of 11.0‰ was

comparable to 10.3‰, which was previously obtained for Atlantic salmon (Molkentin, et al., 2007). This approximate equivalence is confirmed by the fatty acid analysis as well (section 3.3.1.). Although wild Atlantic salmon may be less well distinguishable from conventional Atlantic salmon by $\delta^{15}N_{DDM}$ than Pacific salmon, the combination of $\delta^{15}N_{DDM}$ and $\delta^{13}C_{DDM}$ (**Fig. 1**) probably would still be suitable.

3.2.2. Trout

The analyses of brown trout (*Salmo trutta*) did not include wild stocks but instead only fish from organic and conventional aquaculture. Because no significant differences were detected between raw and smoked samples, the stable isotope data were combined for organic and conventional fish, respectively (**Table 3**). On average, $\delta^{15}N_{DDM}$ as well as $\delta^{13}C_{DDM}$ are higher in organic trout, independent of the smoking process (**Fig. 3**). Hence, the relationship between $\delta^{15}N_{DDM}$ and $\delta^{13}C_{DDM}$ found for trout was similar to that found for salmon (**Fig. 3**), although the correlation between both parameters is less distinct here ($r = 0.70$). Nevertheless, a complete differentiation of both production types was achieved using combined DDM data only, which again chiefly comes from the requirement of a 40% minimum content of animal ingredients in organic aquaculture feed for trout. A comparable differentiation was found for farmed rainbow trout (*O. mykiss*) fed a fish-protein-based or a plant-protein-based diet, respectively (Moreno-Rojas, et al., 2008).

To create a single measure for the authentication of organic trout being suitable for easy use in the practice of food control, the sum of $\delta^{15}N_{DDM}$ and $\delta^{13}C_{DDM}$ was calculated. According to **Table 3**, this $\Sigma\delta_{DDM}$ varied between -14.17 and -10.85‰ in conventional trout and between -10.54 and -6.62‰ in organic trout. Consequently, a minimum $\Sigma\delta_{DDM}$ of -10.7‰ may be set as a limit for organic trout.

The wider distribution of trout samples (**Fig. 3**) compared with salmon (**Fig. 1**) is most likely caused by the differing isotopic environment of individual trout ponds, which provide additional natural feed resources as well, in contrast to the more constant conditions in salmon sea cages. Nevertheless, the average isotopic composition of both salmon and trout DDM is quite similar: for organic and conventional products, approximately 11‰ and 9‰ for $\delta^{15}N_{DDM}$ and approximately -20‰ and -22‰ for $\delta^{13}C_{DDM}$, respectively. While a similar relative shift between organic and conventional samples was found for the average $\delta^{13}C_{LIP}$ in salmon (**Table 2**), the $\delta^{13}C_{LIP}$ in trout was nearly equivalent in both production types (**Table**

3). The latter finding indicates that the fatty acids in trout lipids primarily originated from the fat fraction of the feed, which showed similar $\delta^{13}C_{LIP}$ values in both production types (**Table 4**). Apparently all feeds contained a similar ratio of fish oil to vegetable oil.

Moreover, the differences between organic and conventional trout fillets recorded in the DDM isotopic composition also basically corresponded to the δ-values of the feed (**Table 4**). The $\delta^{15}N_{DDM}$ in organic feed (mean = 9.3‰) was markedly higher than in conventional feed (mean = 4.9‰). The organic feed also showed $\delta^{13}C_{DDM}$ values that were higher by 2.2‰ on average. These differences in feed composition in organic aquaculture are primarily caused by a higher percentage of animal protein or the use of animal protein from a higher food chain level.

The inconsistent results obtained for $\delta^{13}C_{DDM}$ and $\delta^{13}C_{LIP}$ in trout provide an alternative method for the authentication of organic fish. Combining both parameters also facilitated the differentiation of conventional and organic trout (**Fig. 4**). However, this procedure requires more analytical effort because it requires two separate analyses. Nonetheless, it allows the calculation of a single parameter ($\Delta\delta^{13}C$) that indicates the organic origin without any further comparative samples. As **Table 3** shows, $\Delta\delta^{13}C$ varied between 3.63 and 5.94‰ in conventional trout and between 6.23 and 7.73‰ in organic trout. Hence, a minimum $\Delta\delta^{13}C$ of 6.0‰ may be set as a limit for organic trout.

The subgroups occurring particularly in **Fig. 3** mainly represent clusters of samples obtained from a specific farm, whereas the resemblance of farms E and F resulted from using the same feed. Sometimes different sampling years separated within these clusters (farms B and F) but the fish from farm D obtained at different dates resulted in distinctly separate clouds. This indicates the variability in feed composition as well as in environmental conditions and their influence on the analytical results. Although the empirical limits established in this study are currently applicable for the authentication of organic fish, these may change over time with modified feeding practices and, hence, have to be checked and possibly adapted in future.

3.3. Fatty acids

3.3.1. Salmon

The type of processing did not cause significant differences in the fatty acid composition of salmon lipids. Hence, **Table 5** shows the combined data of the graved and smoked products, though they are separated according to the type of production. The most prominent difference between the production types was the linoleic acid (18:2n6) content, which allowed clear distinction of all three origins. As shown in **Fig. 5b**, the 18:2n6 ranges of the wild, organic and conventional salmon samples were distinctly different and showed no overlap. The lowest 18:2n6 contents were found in wild salmon and the highest in conventional salmon. This order clearly corresponds to the increasing amount of ingested vegetable oils, which are rich in linoleic acid. In principle, the influence of dietary fatty acids on salmon lipids has previously been documented (Ackman & Takeuchi, 1986), although not in relation to differences between conventional and organic aquaculture. However, considerably higher 18:2n6 contents in farmed compared to wild *S. salar* have repeatedly been reported (Ackman & Takeuchi, 1986; Aursand, et al., 2000; Thomas, et al., 2008; van Vliet & Katan, 1990).

As mentioned above, one sample of smoked salmon declared as organic had a strikingly high 18:2n6 content of 11.48%, which clearly falls into the conventional range. Because isotope and carotenoid analysis showed corresponding evidence, this sample was rated as a false declaration and, thus, excluded from **Table 5** and **Fig. 5**. Therefore, according to the present results, the authentication of organic salmon can be achieved by only one analysis of the fatty acid composition, whereas authentication by stable isotopes requires two separate analyses if wild fish is included (section 3.2.1.).

In contrast to our previous salmon study (Molkentin, et al., 2007), which determined that a combination of $\delta^{15}N$ and 18:2n6 content was required to distinguish the three production types, the present results showed that now only 18:2n6 was needed. While the 18:2n6 range and average in the wild (1.45 – 2.35%, avg. 1.88%) and organic (5.75 – 7.95%, avg. 6.53%) salmon from the previous study agree with the current data (**Table 5**), the previous wide range of 4.03 – 10.65% (avg. 6.40%) for 18:2n6 in conventional salmon has now been narrowed down to 10.79 – 13.00%, a markedly elevated level. As observed with the decreased δ-values of conventional salmon (section 3.2.1.), this finding indicates that the percentage of animal feed

components has already been minimised in all conventional aquaculture farms. Consequently, the percentage of vegetable oil, which contains abundant 18:2n6, is consistently high in conventional feed. This extreme composition is not allowed in the organic aquaculture of carnivores but is feasible in conventional salmon farming (Torstensen, et al., 2008).

The different feed composition in conventional and organic aquaculture can also be recognised by elevated contents of oleic acid and linolenic acid in conventional salmon (**Table 5**). These fatty acids, common in vegetable oils, also clearly discriminated conventional salmon from organic and wild salmon (**Fig. 5c**). A prerequisite for distinguishing production types by a single parameter is that there is no overlap of data ranges. Correspondingly, conventional salmon showed distinctly decreased palmitic acid, EPA and DHA contents (**Fig. 5d**) in comparison with organic and wild salmon. However, the highest contents of EPA and DHA, the nutritionally important omega-3 fatty acids, occurred in some samples of wild salmon, which is in accordance with studies comparing wild and conventionally farmed salmon (Ackman & Takeuchi, 1986; van Vliet & Katan, 1990). Moreover, a distinction between organic and conventional salmon could also be achieved by pentadecanoic, stearidonic, eicosadienoic, arachidonic or docosapentaenoic acids, but they did not differentiate organic from wild salmon.

The discrimination between wild and organic salmon was less obvious. Except for the linoleic acid content, wild and organic salmon only differed significantly in myristic acid (14:0) content (**Fig. 5a**). Although reported by Ackman & Takeuchi (1986), 14:0 did not allow a distinction between wild and conventional salmon in the present study. However, its high content in organic salmon is another unique characteristic suitable for authentication. Overall, the differences in the fatty acid composition between the organic and wild salmon were less distinct than between the organic and conventional salmon. Hence, the differentiation from wild salmon is the greater challenge in the authentication of organic salmon.

The lipid contents in salmon fillet (**Table 5**, last line) showed significant differences between farmed and wild ($P < 0.001$), but not between organically and conventionally farmed salmon. Slightly decreased lipid contents of wild compared to farmed salmon have been reported for *S. salar* previously (Bell, et al., 1998; Johnston, et al., 2006), but apparently the deviation is largely due to species specific characteristics of *O. nerka* here. On the other hand, wild *O. nerka* significantly

differed in the protein content (MV ± SD: 23.77 ± 0.78%) only from conventional *S. salar* (21.40 ± 1,24%) but not from organic (22.89 ± 1.70%).

3.3.2. Trout

Similar to salmon, the smoking process did not perceptibly affect the fatty acid composition in trout lipids compared with raw trout. Hence, **Table 6** shows the pooled fatty acid data for the organic and conventional samples. Although samples from wild stocks were not included, both aquaculture variants could not even be discriminated by any single fatty acid. As **Table 6** shows, the ranges of all fatty acids overlapped between the organic and conventional samples. The same applied to the total lipid contents (**Table 6**), although at first view conventional trout samples on average showed significantly lower lipid contents ($P < 0.05$).

There were certain trends that indicated higher contents of all saturated fatty acids and gondoic acid in organic trout but lower contents of oleic acid and linolenic acid than in conventional trout. While oleic acid and linolenic acid are related to vegetable oil sources, gondoic acid originates from zooplankton (Budge, Iverson, Bowen, & Ackman, 2002) and can accumulate in marine food chains. These findings correlate to an expected feed composition with a higher percentage of animal components and a lower percentage of vegetable components in organic aquaculture feed (cp. section 3.2.2.).

Moreover, the increased contents of oleic and linolenic acids as well as the decreased contents of SFA and gondoic acid found in conventional trout lipids are in good agreement with the lipid composition of the corresponding feeds (**Table 7**). However, the elevated average content of linoleic acid (18:2n6) in organic trout lipids, which did not correspond to the results of organic salmon, is somewhat striking because organic feed contained on average less 18:2n6 than conventional feed. Because the feed samples were obtained only twice per aquaculture plant, we may not have recorded the entire compositional feed variation during the lifetime of the fish. Remarkably, the range of 18:2n6 content is particularly wide in organic trout lipids (**Table 6**), indicating that some animals received an increased input of linoleic acid. In fact, the two organic farms (E and F) producing the fish with the highest 18:2n6 content used feed containing more than twice the amount of 18:2n6 compared with the third organic farm. Although organic feed contained more EPA

(20:5n6) and DHA (22:6n3), these nutritionally important fatty acids showed nearly the same content in organic and conventional trout lipids (**Table 6**).

The unambiguous authentication of organic trout products was achieved by the combination of oleic acid and gondoic acid contents. **Fig. 6** shows the correlation of both parameters and demonstrates the complete differentiation of organic and conventional trout. The symbols in **Fig. 6** also indicate smoked samples, which were not perceptibly different from the raw samples, although their distribution may suggest that. However, smoked trout could not be obtained from every farm selling raw trout, which due to the smaller variation seemingly caused a better differentiation for smoked than raw trout.

3.4. Carotenoids

3.4.1. Salmon

Carotenoid content

The degree of flesh pigmentation depends on various endogenous and exogenous factors such as age, weight, stage of maturity and health status of the animals as well as the nature of the feed, the level and the type of carotenoids in the feed and the feeding period. The fact that some salmon species are much more intensely coloured than others is conditioned by genetic constitution (Putnam, 1991). Generally, Pacific salmon (*Oncorynchus spp*.) store carotenoids better than Atlantic salmon (*S. salar*).

The astaxanthin contents of all investigated salmon products are summarised in **Table 8**. As expected, the wild salmon of the species *O. nerka*, which is also called red salmon or sockeye and is characterised by bright red coloured flesh, contained significantly ($P < 0.001$) more astaxanthin than both kinds of farmed salmon (*S. salar*). However, the differentiability primarily is due to different species here. The only exception was one sample produced from *O. kisutch*, which is also known as silver salmon or coho. Within the wild samples of *O. nerka*, graved salmon (MV ± SD: 16.5 ± 5.3 µg astaxanthin/g salmon tissue) on average did contain less astaxanthin than smoked salmon (MV ± SD: 24.5 ± 6.8 µg/g), although the difference proved not significant. The average difference between graved and smoked samples was considerably smaller within organic (3.1 vs. 3.5 µg/g) and conventional salmon (4.1 vs. 3.9 µg/g). Because, over-all differences between organic and conventional

563 salmon were not significant (**Table 8**), the astaxanthin content is not suitable for the
564 authentication of organic salmon.

565 Moreover, the lipid contents did not correlate with the carotenoid concentrations
566 within each species (*S. salar* or *O. nerka*). A high lipid content was not
567 simultaneously connected to an elevated or reduced content of fat-soluble
568 astaxanthin, which is in agreement with previous studies on *S. salar* (Bell, et al.,
569 1998). However, in comparison with *S. salar*, the lower lipid content of wild *O. nerka*
570 coincided with a higher astaxanthin content for environmental and species specific
571 reasons.

572 The canthaxanthin contents were below the detection limit of 0.1 µg/g fish tissue
573 in all wild salmon samples and most samples of both kinds of farmed salmon (**Table**
574 **8**). While two conventionally farmed fish contained small amounts of canthaxanthin,
575 also organically reared salmon partially contained significant amounts. Thus, if
576 canthaxanthin is detected in salmon tissue, it does not have to be conventionally
577 farmed salmon that was fed with a feed containing synthetic canthaxanthin. It can
578 also be organic salmon whose diet contained *Paracoccus carotinifaciens*. This
579 bacterium synthesises astaxanthin as well as canthaxanthin (EFSA, 2007).

580

581 *Configurational astaxanthin isomers*

582 The astaxanthin molecule has two chiral centres. Therefore, it exists as a
583 mixture of three different configurational isomers: two enantiomers, (*3S,3'S*) and
584 (*3R,3'R*), and a *meso*-form, (*3R,3'S*). These forms differ only in the position of the two
585 hydroxyl groups at C-3 and C-3' in the molecule. At these two hydroxyl groups,
586 astaxanthin can be esterified with fatty acids (Olaizola, 2007), but in the muscle of
587 salmonids, astaxanthin exists in the free form (Bjerkeng, 1997). There are no
588 comparable stereoisomers of canthaxanthin.

589 The (*3R,3'R*) : *meso :* (*3S,3'S*) ratio of the three optical isomers depends on the
590 origin of the astaxanthin. Synthetic astaxanthin contains a ratio of 1:2:1, but the red
591 yeast *Phaffia rhodozyma* primarily produces free astaxanthin in the (*3R,3'R*)-
592 configuration. However, other natural sources such as the bacterium *Paracoccus*
593 *carotinifaciens* or the microalgae *Haematococcus pluvialis* predominantly produce the
594 (*3S,3'S*)-isomer (Bjerkeng, 1997; Moretti, et al., 2006; Whyte et al., 1998). In
595 *Thysanoessa inermis* and *Calanus finmarchicus,* two small crustaceans that are

596 important ingredients in the diet of wild salmon, the (*3S,3'S*)-isomer dominates (Foss,
597 Renstrom, & Liaaen-Jensen, 1987; Lura & Sægrov, 1991).

598 Feeding studies with rainbow trout and Atlantic salmon have shown that the
599 relative ratio of the configurational isomers of astaxanthin in the fish muscle
600 corresponds with the isomer distribution in their feed. There is no epimerisation.
601 Salmonids are unable to convert one isomer into another (Storebakken, Foss,
602 Austreng, & Liaaen-Jensen, 1985; Whyte et al., 1998). Therefore, in farmed salmon
603 fed with synthetic astaxanthin the *meso*-form represents approximately 50% of the
604 isomers. This farmed salmon could be clearly distinguished from wild salmon
605 because of its isomer ratio. Various studies have shown that the (*3S,3'S*)-form is the
606 main form found in wild Pacific and Atlantic salmon species. Free-living salmonids
607 contain only relatively low levels of *meso*-astaxanthin. Detection of a high *meso*-
608 astaxanthin content indicates that the fish is most likely a farmed fish (Bjerkeng,
609 1997; Lura & Sægrov, 1991; Ostermeyer & Schmidt, 2004; Schiedt, Leuenberger, &
610 Vecchi, 1981; Turujman, et al., 1997).

611 The ratio of the configurational isomers of all-*trans* astaxanthin in all
612 investigated salmon products is summarised in **Table 8**. In **Figs. 7** and **8**, the
613 percentage distributions of the configurational isomers of astaxanthin found in the
614 graved and smoked salmon products of the present study are pictured.

615 In all graved and smoked products from conventionally farmed Atlantic salmon,
616 the (*3R,3'R*)-, (*3R,3'S*)- and (*3S,3'S*)-isomers were present in an average ratio of
617 24:50:26 (**Fig. 7**). The variations in the percentage ratios were very low between the
618 single products. Thus, *meso*-astaxanthin was the major isomer and both enantiomers
619 were present in comparable amounts, which indicates that the fish feed was
620 exclusively supplemented with synthetic astaxanthin.

621 However, in the fillets of wild Pacific salmon, no *meso*-astaxanthin was detected
622 (i.e., less than 1%) (**Fig. 7**). In these samples, the *SS*-isomer was always the
623 predominant one, averaging 72% with the remaining 28% representing the *RR*-
624 isomer. This is consistent with previously published values (Megdal, Craft, &
625 Handelman, 2009; Turujman, et al., 1997).

626 However, in the tissue of organically farmed Atlantic salmon, the distribution of
627 isomers was very inconsistent (**Fig. 8**). The three Norwegian graved products (NO*)
628 contained almost exclusively *SS*-isomer (MV: 97.1%). For the remainder, only the
629 *RR*-isomer and no *meso*-astaxanthin were detected. In one smoked salmon sample

derived from the North Atlantic (NA), a very similar isomer distribution was found. The diet for these salmon was presumably supplemented with *Paracoccus carotinifaciens*, which would also explain the presence of canthaxanthin in one of the products.

In two Norwegian (NO) and three Irish (IE) smoked samples, the *RR*-isomer (94% - 100%) predominated significantly. The remainder consisted only of the *SS*-isomer. Obviously, these salmon had received feed containing the yeast *Phaffia rhodozyma.* In contrast, one smoked salmon sample from Scotland (GB) had an isomer distribution that corresponded with wild salmon. Furthermore, in two other Irish smoked samples and in a Scottish graved sample (GB*), the *RR*- and *SS*-isomers were present in roughly equal proportions without any *meso*-astaxanthin. Perhaps a mixture of *Phaffia rhodozyma* and *Paracoccus carotinifaciens* was added to their diet. The isomer distribution in the remaining three Irish samples was similar to that of conventionally reared salmon. This distribution can be obtained not only with synthetic astaxanthin but also with the addition of shrimp shells to the diet (Lura & Sægrov, 1991). One Scottish sample (not shown) contained the *RR*-, *RS*- and *SS*-isomers in a ratio of 25:48:26. In line with the results from the stable isotope and fatty acid analyses, this product was made from salmon that was obviously not farmed organically but instead conventionally with a diet containing synthetic astaxanthin.

Thus, conventionally farmed salmon could be clearly distinguished from wild salmon by the configurational astaxanthin isomer ratio (**Fig. 7**). However, an unambiguous assignment based on the analysis of carotenoids is not always possible for organically produced salmon (**Fig. 8**). When these animals received feed supplemented with the yeast *Phaffia rhodozyma* or the bacterium *Paracoccus carotinifaciens*, they differed significantly from the conventionally farmed as well as the free-living salmon. However, if they were fed with shrimp shells, it was impossible to distinguish organically from conventionally farmed salmon. Furthermore, in several organic samples, the *RR*-and *SS*-isomers occurred in distributions that could be interpreted as wild salmon.

3.4.2. Trout

All organically reared brown trout contained neither astaxanthin nor canthaxanthin. Even in the conventionally farmed trout, no canthaxanthin was found, and in many samples, no astaxanthin was detected either.

For one conventional trout farm only, small amounts of astaxanthin were found in all animals. The smoked specimens contained significantly less astaxanthin (MV ± SD: 0.2 ± 0.1 µg/g) than the fresh fish (0.8 ± 0.5 µg/g). The average astaxanthin isomer ratio of 29% *RR* : 45% *RS* : 26% *SS* found in these fish fillets indicates the addition of synthetic astaxanthin to the feed. In some brown trout from two other conventional farms, small amounts of synthetic astaxanthin were also detectable.

Overall, carotenoid analysis was not suitable to identify the production type of brown trout. Unlike salmon, consumers do not expect coloured flesh with brown trout. Hence, colouring feed additives are not crucial in trout farming.

4. Conclusions

The tested analytical methods provided several approaches for authenticating organic salmonid products. In contrast to our previous findings (Molkentin, et al., 2007), both stable isotope as well as fatty acid analysis alone proved suitable to identify organic salmonid products, regardless of processing. While isotopic analyses produced comparable results in salmon and trout, fatty acid differentiators were different between the species. Carotenoid analysis was applicable to salmon only, but could not identify organic fish unambiguously in all cases.

The special feed composition required in organic aquaculture resulted in characteristic changes in the fish tissue. However, differences between organic and wild salmon were less distinct than between organic and conventional. Overall, differences between the production types were related to varying percentages of animal and vegetable components in the diet. Because the composition in particular of conventional aquaculture feed may be subject to fluctuations, the currently established limits for the analytical differentiators have to be checked and possibly adapted in future.

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819

Figure Captions

Fig. 1: $\delta^{15}N_{DDM}$ and $\delta^{13}C_{DDM}$ in processed salmon fillets from different origins

Fig. 2: $\delta^{15}N_{DDM}$ and $\delta^{13}C_{LIP}$ in smoked and graved salmon fillets from different origins

Fig. 3: $\delta^{15}N_{DDM}$ and $\delta^{13}C_{DDM}$ in raw and smoked brown trout fillets from different origins; capital letters denote farm lables (see Table 1)

Fig. 4: $\delta^{13}C_{DDM}$ and $\delta^{13}C_{LIP}$ in raw and smoked brown trout fillets from different origins

Fig. 5: Variation (minimum, maximum, 25th and 75th percentile, median) of fatty acids in processed salmon fillets from different origins: a) 14:0, b) 18:2n6, c) 18:3n3, d) 22:6n3

Fig. 6: Content of oleic (18:1n9) and gondoic (20:1n9) acids in raw and smoked brown trout fillets from different origins

Fig. 7: Percentage distribution of configurational isomers of all-*trans* astaxanthin [(*3R,3'R) = RR; (3R,3'S) = RS;* (*3S,3'S*) = SS] in smoked or graved products from conventionally farmed *(S. salar)* and wild salmon *(O. nerka, *O. kisutch)*

Fig. 8: Percentage distribution of configurational isomers of all-*trans* astaxanthin in smoked or graved (*) products from organically farmed salmon (GB = Scotland; IE = Ireland; NA = North Atlantic; NO = Norway)

Table 1: Number and declared origin of wild and farmed fish samples

		Wild	Organically farmed	Conventionally farmed
Salmon	species	*Oncorhynchus nerka* (n=20), *Oncorhynchus kisutch* (n=1)	*Salmo salar*	*Salmo salar*
	smoked (s)	11	12	10
	graved (g)	10	5	10
	producers (s / g)	6 / 2	8 / 3	6 / 6
	origin	Alaska	Ireland, Norway, Scotland	Norway
Trout	species	–	*Salmo trutta*	*Salmo trutta*
	raw (r)	–	25	30
	smoked (s)	–	7	10
	producers (r / s)	–	3 / 2	3 / 2
	farm label (r / s)	–	D, E, F / D, F	A, B, C / A, C
	origin	–	Germany	Germany

Table 2: Stable isotope composition of processed salmon (MV ± SD: mean value ± standard deviation; Min: minimum, Max: maximum; n: number of products)

Production	n	$\delta^{15}N_{DDM}$ (‰)	$\delta^{13}C_{DDM}$ (‰)	$\delta^{13}C_{LIP}$ (‰)	
wild	21	11.04 ± 0.48	-20.37 ± 0.37	-27.85 ± 0.59	**MV ± SD**
		10.43	-21.08	-28.71	**Min**
		12.55	-19.83	-26.63	**Max**
organic	16[a)]	11.61 ± 0.83	-19.68 ± 0.62	-25.96 ± 0.45	**MV ± SD**
		10.62	-20.45	-26.55	**Min**
		13.45	-18.78	-24.92	**Max**
conventional	20	8.92 ± 0.57	-21.98 ± 0.55	-27.66 ± 0.26	**MV ± SD**
		7.93	-22.64	-28.17	**Min**
		9.80	-20.22	-26.99	**Max**

[a)] one out of 17 samples was excluded; see text

Table 3: Stable isotope composition of raw and smoked brown trout (MV ± SD: mean value ± standard deviation; Min: minimum, Max: maximum; n: number of products)

Production	n	$\delta^{15}N_{DDM}$ (‰)	$\delta^{13}C_{DDM}$ (‰)	$\delta^{13}C_{LIP}$ (‰)	$\Delta\delta^{13}C$ (‰)	$\Sigma\delta_{DDM}$ (‰)	
organic	32	11.59 ± 0.87	-20.46 ± 0.72	-27.19 ± 0.37	6.74 ± 0.39	-8.87 ± 1.08	**MV ± SD**
		10.02	-21.23	-27.74	6.23	-10.54	**Min**
		12.83	-18.92	-26.57	7.73	-6.62	**Max**
conventional	40	9.29 ± 0.50	-22.03 ± 0.62	-27.11 ± 0.54	5.08 ± 0.56	-12.74 ± 0.97	**MV ± SD**
		8.50	-23.07	-28.31	3.63	-14.17	**Min**
		10.28	-21.12	-26.26	5.94	-10.85	**Max**

Table 4: Stable isotope composition of brown trout aquaculture feed

Production	Farm	$\delta^{15}N_{DDM}$ (‰)	$\delta^{13}C_{DDM}$ (‰)	$\delta^{13}C_{LIP}$ (‰)	$\Delta\ \delta^{13}C$ (‰)
conventional	A	5.58	-24.64	-27.64	3.00
	B	4.71	-23.57	-27.23	3.66
	C	4.54	-24.86	-27.58	2.73
organic	D	9.89	-21.17	-26.70	5.53
	E, F	8.70	-23.05	-27.50	4.45

Table 5: Average composition of fatty acids in processed salmon from different origins (% 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		**wild**	**organic**	**conventional**
		n=21	n=16	n=20
Common name	Code	**MV ± SD Min - Max**		
Myristic acid	14:0	4.85 ± 0.70 3.67 - 5.99	7.31 ± 0.79 6.09 - 8.67	4.09 ± 0.43 3.47 - 5.06
Pentadecanoic acid	15:0	0.45 ± 0.09 0.16 - 0.58	0.54 ± 0.03 0.49 - 0.60	0.29 ± 0.04 0.23 - 0.38
Palmitic acid	16:0	20.91 ± 1.39 18.43 - 23.25	18.79 ± 0.89 17.04 - 19.85	12.84 ± 0.85 11.83 - 14.86
Heptadecanoic acid	17:0	n.d.	n.d.	n.d.
Stearic acid	18:0	4.07 ± 0.51 3.06 - 5.10	3.66 ± 0.37 3.12 - 4.39	2.94 ± 0.18 2.70 - 3.39
∑SFA		30.28 ± 1.55 27.03 - 32.68	30.3 ± 1.54 27.61 - 32.47	20.17 ± 1.41 18.48 - 23.55
Palmitoleic acid	16:1n7	5.67 ± 1.45 3.41 - 8.69	7.21 ± 1.17 5.52 - 9.50	4.45 ± 0.48 3.83 - 5.61
Oleic acid	18:1n9	20.11 ± 2.99 15.72 - 26.61	16.97 ± 1.22 15.05 - 19.60	36.03 ± 2.00 30.94 - 38.71
Vaccenic acid	18:1n7c	4.71 ± 0.83 2.90 - 5.92	3.23 ± 0.31 2.83 - 3.82	3.44 ± 0.10 3.31 - 3.70
Gondoic acid	20:1n9	6.27 ± 1.56 4.11 - 9.43	7.28 ± 1.43 4.94 - 9.68	4.47 ± 0.67 3.79 - 6.92
∑MUFA		36.77 ± 5.64 26.57 - 47.42	34.69 ± 1.35 32.33 - 37.18	48.4 ± 1.44 44.36 - 50.49
Linoleic acid	18:2n6	2.25 ± 0.45 1.52 - 3.58	6.81 ± 1.00 5.49 - 8.88	12.12 ± 0.55 10.79 - 13.00
γ-Linolenic acid	18:3n6	n.d.	n.d.	n.d.
α-Linolenic acid	18:3n3	1.08 ± 0.32 0.26 - 1.66	1.77 ± 0.21 1.38 - 2.14	4.70 ± 0.40 3.91 - 5.39
Stearidonic acid	18:4n3	1.48 ± 0.48 0.52 - 2.41	2.30 ± 0.23 1.89 - 2.71	1.01 ± 0.13 0.79 - 1.39
Eicosadienoic acid	20:2n6	0.53 ± 0.10 0.35 - 0.71	0.58 ± 0.09 0.41 - 0.69	0.95 ± 0.09 0.76 - 1.15
Arachidonic acid	20:4n6	0.64 ± 0.10 0.44 - 0.80	0.73 ± 0.09 0.59 - 0.88	0.40 ± 0.05 0.29 - 0.48
Eicosapentaenoic acid EPA	20:5n3	8.54 ± 1.22 6.27 - 11.29	8.19 ± 1.02 6.15 - 10.46	4.36 ± 0.44 3.49 - 5.58
Docosatetraenoic acid	22:4n6	n.d.	n.d.	n.d.
Docosapentaenoic acid DPA	22:5n3	2.34 ± 0.38 1.89 - 2.97	3.03 ± 0.29 2.52 - 3.57	2.03 ± 0.21 1.66 - 2.37
Docosahexaenoic acid DHA	22:6n3	16.09 ± 4.09 9.55 - 23.13	11.59 ± 1.61 9.38 - 14.91	5.87 ± 0.64 4.70 - 7.18
∑PUFA		32.95 ± 5.10 25.44 - 41.61	35.01 ± 1.39 32.64 - 37.24	31.44 ± 0.99 28.86 - 33.64
EPA + DHA		24.63 ± 4.52 16.29 - 31.64	19.78 ± 1.33 17.07 - 22.33	10.23 ± 0.99 8.20 - 12.45
Total lipids (% of fillet)		3.06 ± 1.53 1.58 - 7.34	8.74 ± 1.53 5.91 - 11.94	9.54 ± 1.88 7.02 - 14.01

n.d. = not detected

Table 6: Average composition of fatty acids in raw and smoked trout from different origins (% of fatty acids measured; for abbreviations see Table 5)

Fatty acid		**organic**	**conventional**
		n=32	n=40
Common name	Code	MV ± SD Min - Max	
Myristic acid	14:0	4.53 ± 1.77 2.21 - 6.78	3.29 ± 0.95 1.81 - 4.72
Pentadecanoic acid	15:0	0.38 ± 0.14 0.20 - 0.57	0.27 ± 0.05 0.16 - 0.36
Palmitic acid	16:0	16.64 ± 2.3 12.97 - 20.89	14.49 ± 2.44 10.13 - 18.68
Heptadecanoic acid	17:0	n.d	n.d
Stearic acid	18:0	3.43 ± 0.25 2.96 - 3.91	2.87 ± 0.20 2.49 - 3.33
∑SFA		24.98 ± 4.19 19.30 - 31.98	20.91 ± 3.37 14.76 - 26.46
Palmitoleic acid	16:1n7	4.41 ± 0.93 3.02 - 6.04	4.65 ± 1.34 2.51 - 7.25
Oleic acid	18:1n9	21.29 ± 3.10 17.77 - 26.47	30.44 ± 4.53 22.96 - 38.72
Vaccenic acid	18:1n7c	2.81 ± 0.30 2.25 - 3.25	3.14 ± 0.20 2.79 - 3.71
Gondoic acid	20:1n9	5.52 ± 1.46 3.12 - 7.65	3.23 ± 0.78 1.63 - 4.99
∑MUFA		34.04 ± 3.00 27.72 - 38.37	41.45 ± 4.27 33.47 - 49.35
Linoleic acid	18:2n6	14.55 ± 6.20 7.20 - 23.25	11.45 ± 1.49 8.85 - 15.29
γ-Linolenic acid	18:3n6	n.d.	n.d.
α-Linolenic acid	18:3n3	2.02 ± 0.27 1.56 - 2.46	2.90 ± 0.56 1.60 - 3.66
Stearidonic acid	18:4n3	1.71 ± 0.77 0.80 - 2.87	1.33 ± 0.31 0.81 - 1.88
Eicosadienoic acid	20:2n6	0.78 ± 0.20 0.51 - 1.35	0.60 ± 0.15 0.43 - 1.15
Arachidonic acid	20:4n6	0.79 ± 0.18 0.49 - 1.07	0.71 ± 0.18 0.43 - 1.07
Eicosapentaenoic acid EPA	20:5n3	3.84 ± 1.09 2.24 - 5.25	4.34 ± 1.08 2.28 - 6.47
Docosatetraenoic acid	22:4n6	n.d.	n.d.
Docosapentaenoic acid DPA	22:5n3	1.74 ± 0.32 1.11 - 2.13	1.68 ± 0.43 0.78 - 2.42
Docosahexaenoic acid DHA	22:6n3	15.56 ± 3.48 9.21 - 22.07	14.62 ± 2.40 9.91 - 19.57
∑PUFA		40.98 ± 5.23 32.70 - 50.79	37.63 ± 2.78 31.7 - 42.56
EPA + DHA		19.40 ± 4.11 11.52 - 25.88	18.96 ± 3.06 13.08 - 25.47
Total lipids (% of fillet)		5.68 ± 2.10 2.50 - 9.29	4.66 ± 1.41 1.96 – 8.02

n.d. = not detected

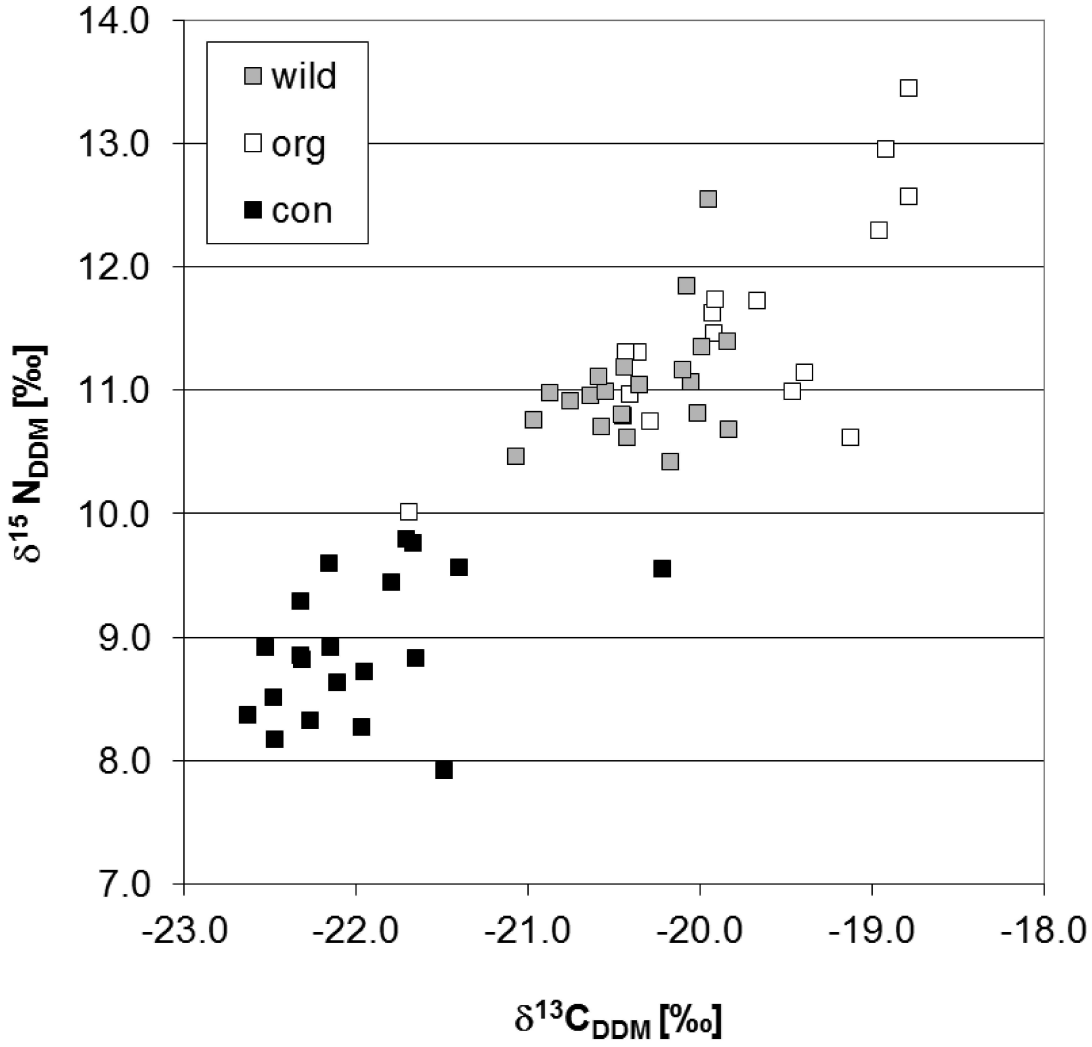
Table 7: Average composition of fatty acids in aquaculture feed from different trout farms (% of fatty acids measured; for abbreviations see Table 5)

Fatty acid		organic	conventional
		n=3	n=3
Common name	Code	MV ± SD Min - Max	
Myristic acid	14:0	7.11 ± 1.41 6.29 - 8.74	3.80 ± 0.64 3.34 - 4.53
Pentadecanoic acid	15:0	0.40 ± 0.21 0.28 - 0.64	0.14 ± 0.09 0.07 - 0.24
Palmitic acid	16:0	18.12 ± 1.59 17.20 - 19.96	18.88 ± 11.36 11.95 - 31.99
Heptadecanoic acid	17:0	0.14 ± 0.12 0.00 - 0.21	0.11 ± 0.07 0.07 - 0.19
Stearic acid	18:0	3.13 ± 0.17 3.03 - 3.32	2.79 ± 0.66 2.29 - 3.54
∑SFA		28.89 ± 3.26 27.01 - 32.66	25.72 ± 12.80 17.98 - 40.49
Palmitoleic acid	16:1n7	5.64 ± 0.25 5.49 - 5.93	4.4 ± 0.68 3.83 - 5.15
Oleic acid	18:1n9	19.65 ± 2.92 16.28 - 21.34	34.37 ± 7.02 26.31 - 39.13
Vaccenic acid	18:1n7c	2.73 ± 0.02 2.72 - 2.76	2.57 ± 0.69 1.77 - 2.97
Gondoic acid	20:1n9	6.03 ± 1.74 5.02 - 8.04	2.61 ± 0.36 2.33 - 3.02
∑MUFA		34.05 ± 0.90 33.01 - 34.57	43.95 ± 7.36 35.56 - 49.33
Linoleic acid	18:2n6	14.65 ± 5.00 8.88 - 17.54	14.88 ± 5.07 9.44 - 19.47
γ-Linolenic acid	18:3n6	0.06 ± 0.05 0.00 - 0.09	n.d.
α-Linolenic acid	18:3n3	2.58 ± 0.59 1.90 - 2.92	4.28 ± 2.50 1.41 - 5.97
Stearidonic acid	18:4n3	2.76 ± 0.93 2.22 - 3.83	1.47 ± 0.23 1.26 - 1.72
Eicosadienoic acid	20:2n6	0.35 ± 0.01 0.35 - 0.36	0.16 ± 0.06 0.09 - 0.20
Arachidonic acid	20:4n6	0.57 ± 0.09 0.52 - 0.67	0.29 ± 0.05 0.24 - 0.34
Eicosapentaenoic acid EPA	20:5n3	7.42 ± 0.35 7.22 - 7.82	4.62 ± 0.69 3.96 - 5.34
Docosatetraenoic acid	22:4n6	n.d.	n.d.
Docosapentaenoic acid DPA	22:5n3	1.15 ± 0.14 1.07 - 1.31	0.51 ± 0.07 0.43 - 0.56
Docosahexaenoic acid DHA	22:6n3	7.52 ± 1.78 6.49 - 9.58	4.11 ± 0.81 3.37 - 4.98
∑PUFA		37.06 ± 2.35 34.35 - 38.42	30.33 ± 5.73 23.96 - 35.06
EPA + DHA		14.94 ± 2.13 13.71 - 17.40	8.73 ± 1.38 7.92 - 10.32

n.d. = not detected

Table 8: Carotenoid content and percentage ratio of the configurational isomers of all-*trans* astaxanthin in salmon products (MV ± SD: mean value ± standard deviation; Min: minimum, Max: maximum; n: number of products)

	Conventionally farmed salmon			**Organically farmed salmon**			**Wild salmon**		
Salmon species	*Salmo salar*			*Salmo salar*			*Oncorhynchus nerka* 1x *Oncorhynchus kisutch*		
Origin	Norway			Ireland, Scotland, Norway, North Atlantic			Alaska		
n	20			16			21		
	Astaxanthin (µg/g fish flesh)								
	Min	MV ± SD	Max	Min	MV ± SD	Max	Min	MV ± SD	Max
All-*trans* Astaxanthin	2.43	4.03 ± 1.03	5.80	1.03	3.35 ± 1.95	7.70	7.00 (*O. kisutch*: 6.63)	20.49 ± 7.18 (w/o *O. kisutch*)	38.75
	Astaxanthin isomers (%)								
	Min	MV ± SD	Max	Min	MV ± SD	Max	Min	MV ± SD	Max
3R,3`R-Isomer	23.4	24.2 ± 0.4	24.9	1.8	51.0 ± 37.6	100	14.4	28.0 ± 7.9	47.0
3R,3`S-Isomer	49.1	49.7 ± 0.3	50.3	≤ 1%	7.4 ± 16.3	47.2		≤ 1	
3S,3`S-Isomer	25.6	26.1 ± 0.3	26.8	≤ 1%	41.6 ± 37.8	98.2	53.0	72.0 ± 7.9	85.6
	Canthaxanthin (µg/g fish flesh)								
	Min - Max			Min - Max			Min - Max		
Canthaxanthin	< 0.1 (in 18 samples) 0.1 – 0.18 (in 2 samples)			< 0.1 (in 10 samples) 0.1 – 1.38 (in 6 samples)			< 0.1 (all samples)		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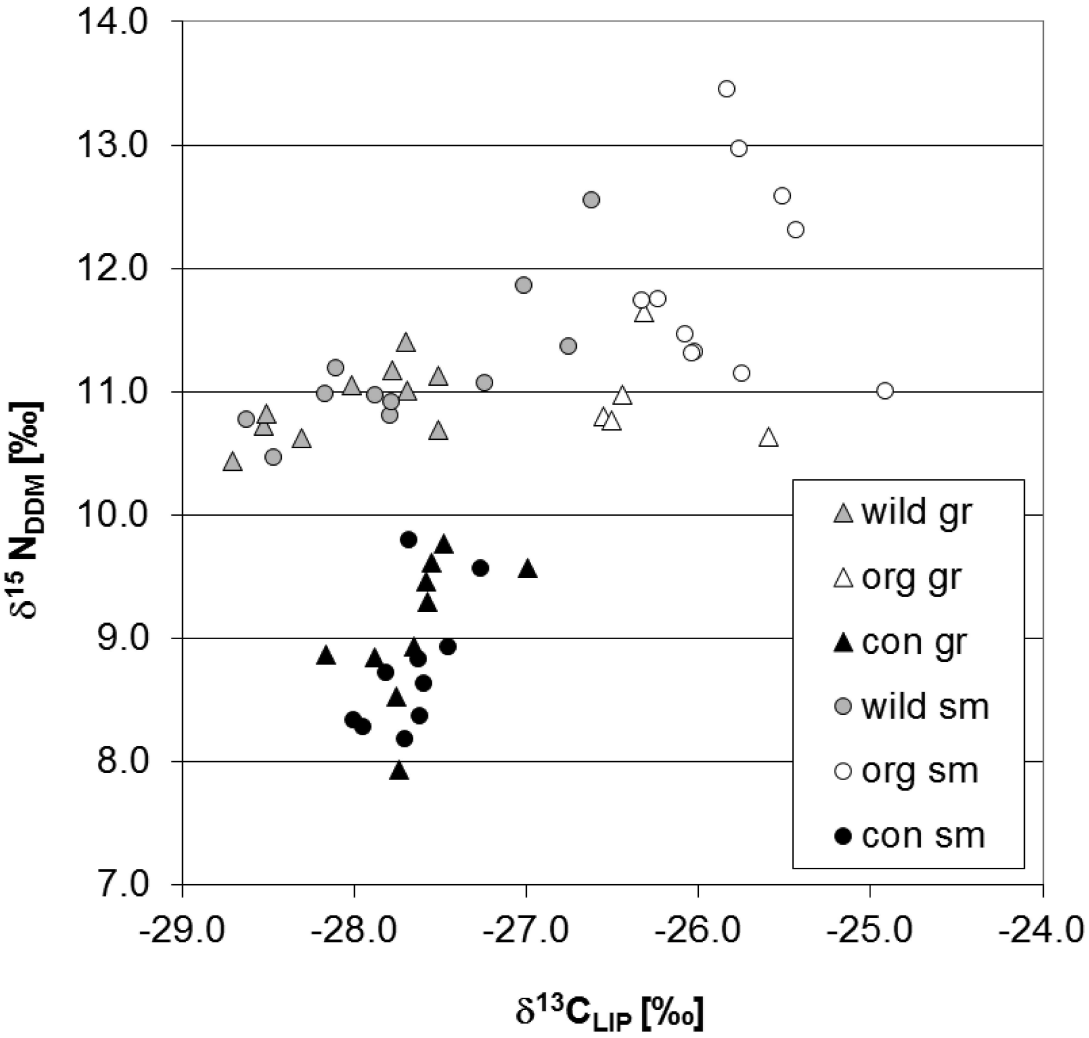
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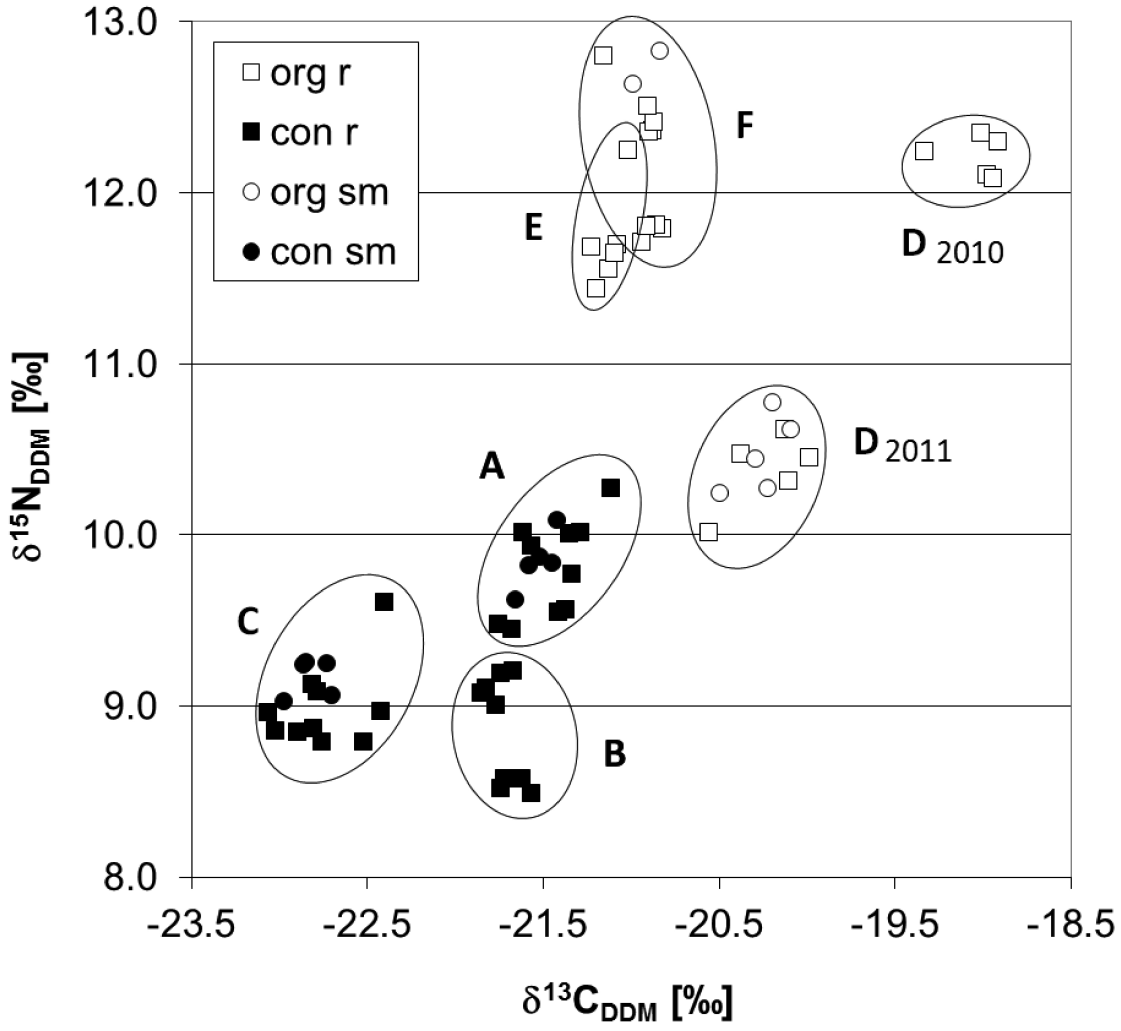
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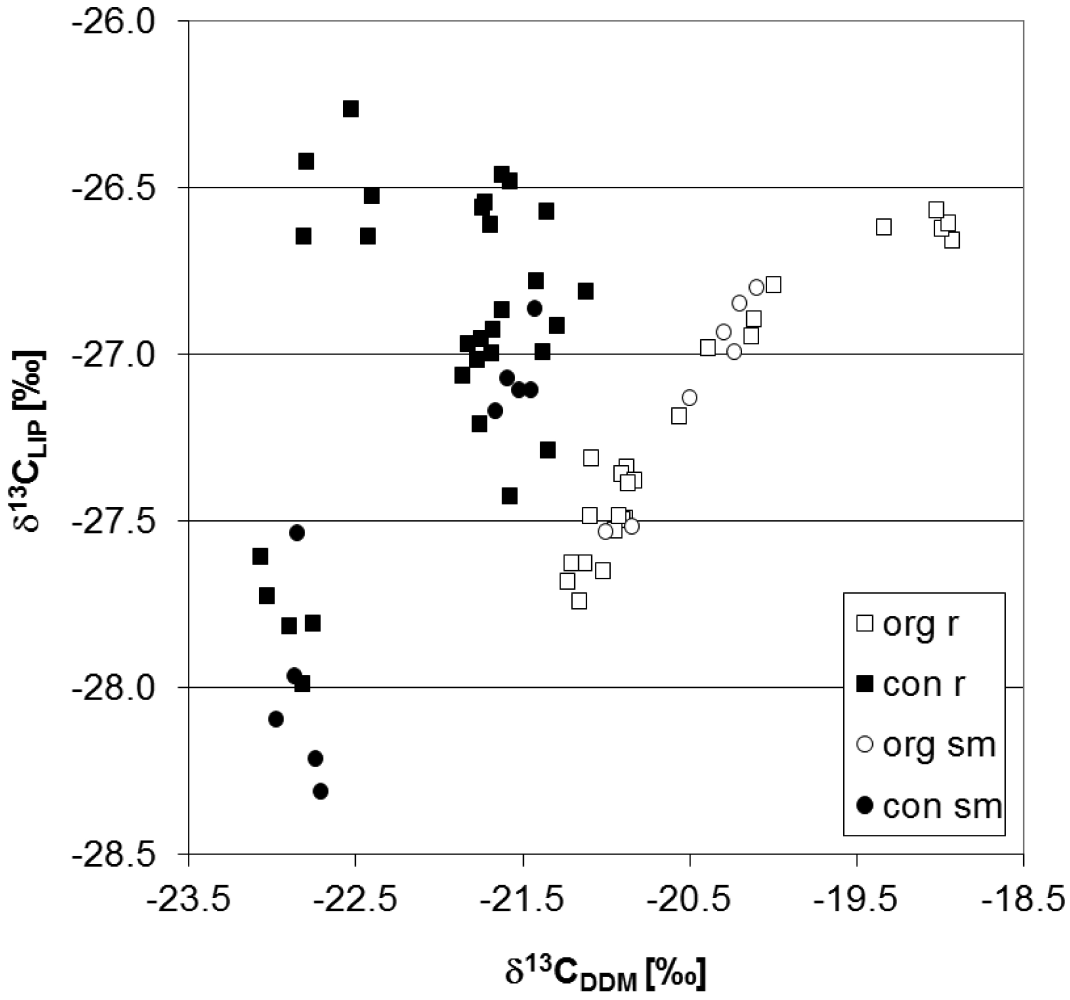
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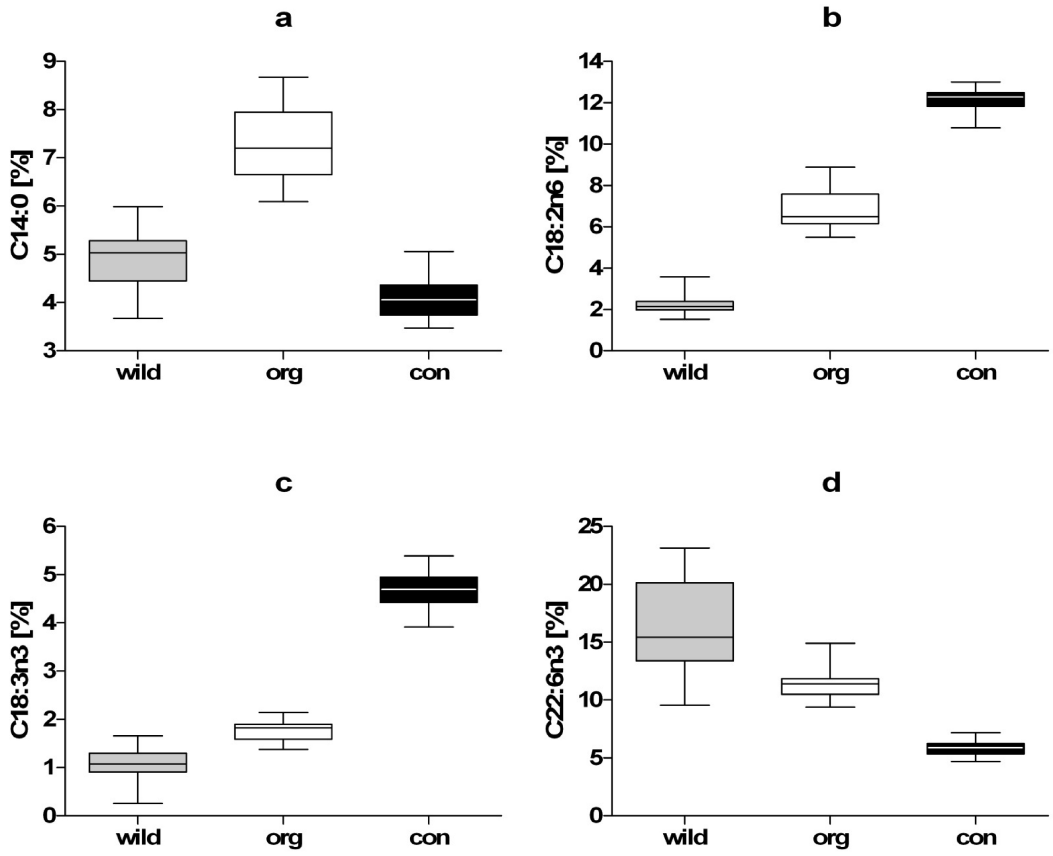
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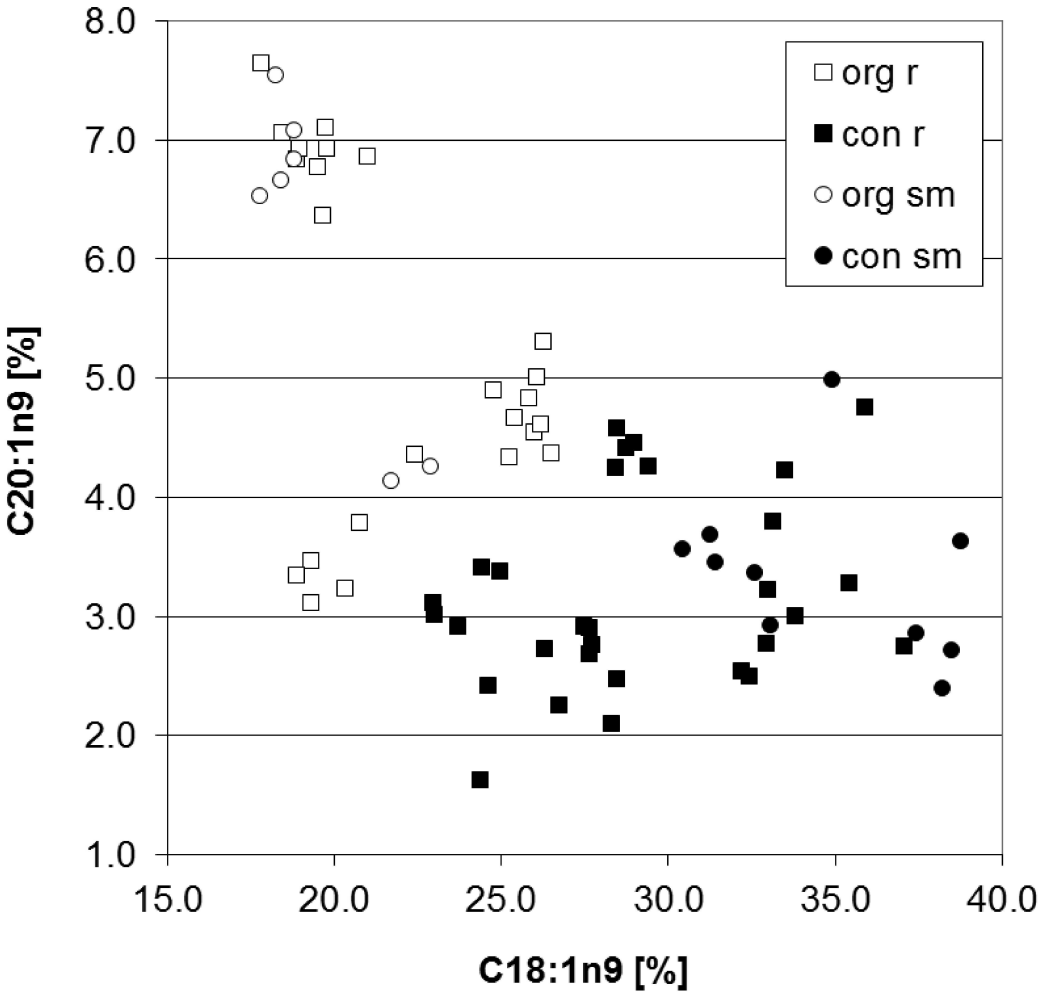
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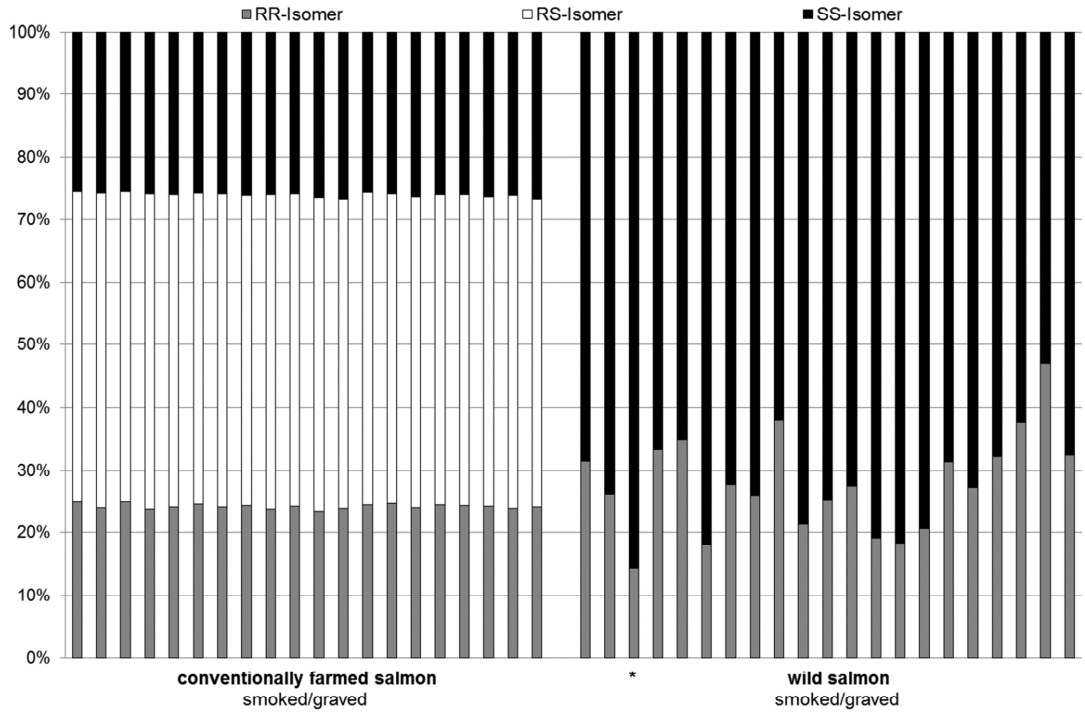

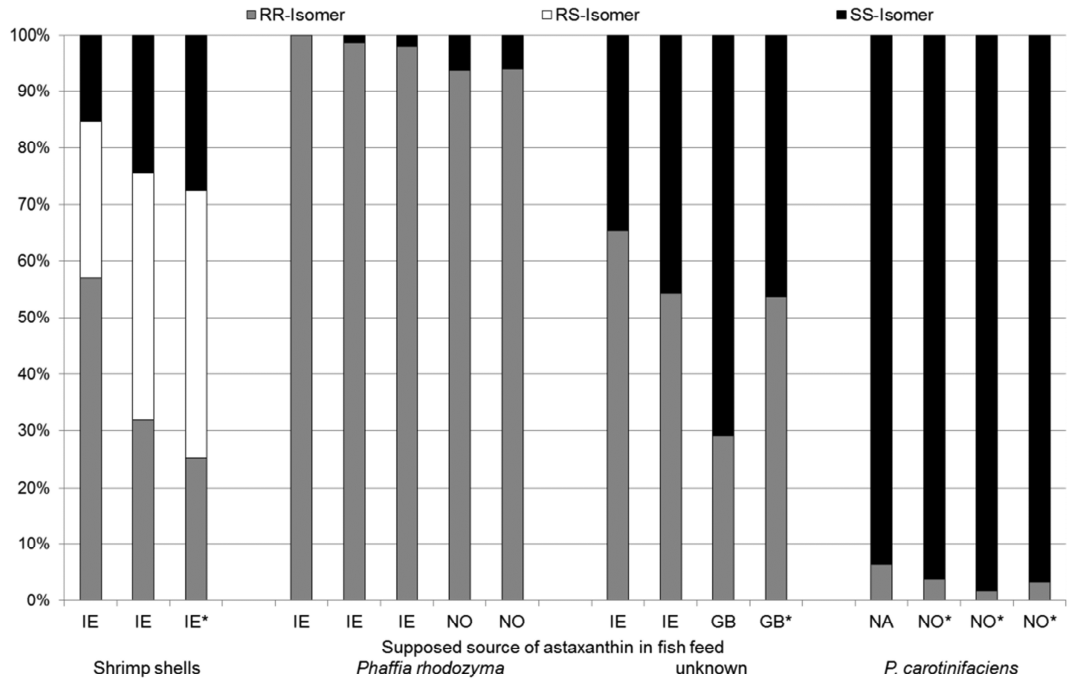

Highlights

- Organic salmonids were authenticated by either stable isotopes or fatty acids
- Organic salmon and trout had higher $\delta^{15}N$ and non-lipid $\delta^{13}C$ than conventional
- Organic salmon showed a linoleic acid content between wild and conventional
- Organic trout was distinguished from conventional by oleic and gondoic acid
- Authentication worked for raw, smoked and graved fish