Project brief

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CLEAN FISH – alternative food from cell culture

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- **Fatty acid pattern from cell culture showed significant differences compared to common fish.**
- **Omega-3-fatty acids EPA and DHA could be detected in fish cells made in the lab.**
- **Fish cells from the lab were less contaminated with mercury than common fish.**

Background and aim

Owing to long-chain omega-3-fatty acids such as EPA and DHA, which had health beneficial properties, fish is known as a healthy food resource.

Since the population in the world is constantly growing, the need for food and so for fish is increasing, too. *In vitro* products from the lab could be one alternative to serve such a demand. As part of the CLEAN FISH project fish cells were grown in the laboratory. For a proper characterisation and comparison to commonly consumed fish, lipid classes and fatty acid patterns were analysed. In addition, contamination with the toxic heavy metal mercury as well as basic ingredients were determined too.

Materials and methods

Five different fish species (herring, rainbow trout, Alaska pollock, chum salmon, atlantic salmon), that have big relevance for the German food market and shrimp were selected for comparison reasons. Methods for the determination of the basic ingredients (water, lipid and ash), mercury contamination, lipid classes and fatty acid patterns were adapted or newly developed and used to analyse the fish samples from the comparison group as well as fish cells of chinook salmon and rainbow trout grown in cell culture, that we obtained from our cooperation partner.

Results

Water and ash contents were in comparable ranges for all samples from the comparison group (80 % and 1 %), whereas lipid contents showed expected differences, since lean and fatty fish species were both included. Cells from the lab consisted of 95 % water and had less than 1 % lipid content related to their wet weight, which is in ranges similar to the lean fish samples such as Alaska pollock or to shrimp (Table 1).

Big differences were observed in the composition of the lipid classes for the fish samples of the comparison group. In this case, fatty species had high proportions of neutral lipid classes, which were mainly triglycerides, that are used as energy reserves in nature. Decreasing fat content was accompanied with an increase of the percentages of the polar fraction, up to 20 % for the lean fish species. Shrimp was an exception here, owing 60 % polar lipids. Since the neutral fraction consists mostly of cholesterol, almost no lipids classes for energy storage purposes could be detected in the shrimp samples.

Table 1: Mercury and Lipid contents and percentages of neutral (NL) and polar lipid (PL) fractions of the total lipid. Species: atlantic almon (*Salmo salar*); chum salmon (*Oncorhynchus keta*); Alaska pollock (*Gadus chalcogrammus*); rainbow trout (*Oncorhynchus mykiss*); herring (*Clupea harengus*); white shrimp (*Litopenaeus vannamei*); cell culture: chinook salmon (*Oncorhynchus tshawytscha*); rainbow trout (*Oncorhynchus mykiss*).

Species	Mercury [µg/kg]	Lipid [%]	NL [%]	PL [%]
atlantic salmon	17.85-24.74	3.80-18.32	91.60-96.68	1.40-3.58
chum salmon	23.79-39.20	0.85-2.21	76.41-88.19	6.72-16.66
Alaska pollock	8.13-13.52	0.66-1.33	76.49-81.28	16.20-19.72
rainbow trout	8.50-63.11	4.85-13.45	30.72-98.33	0-4.26
herring	51.39-92.75	4.13-12.23	86.79-92.54	0.71-3.91
shrimp	3.82-16.46	0.94-1.08	29.07-34.04	65.95-67.71
cell culture chinook salmon	2.40/9.93	0.57/0.72	26.81/ 30.29	68.70/ 73.19
cell culture rainbow trout	-	0.99	18.87	75.58

Interestingly, we did not find similarities in the lipid classes from the cultured cell to the fish species of the comparison group, but we did find some to shrimp. Here we observed comparable proportions of polar lipid fractions and cholesterol. Unsurprisingly, we found different contamination levels with mercury depending on the various species. Samples from cell culture had low mercury contents, which were in the range of the lowest measured values for samples of the comparison group. All measured values were clearly below the limit of 500 µg per kg, that is given by the EU for fish as food resource.

We developed a gas chromatographic method for the analysis of fatty acid methyl esters (FAME), that was highly sensitive especially for middle and long chain derivatives. Using this method, we were able to create detailed fatty acid patterns of the samples of the comparison group as well as for the cultured cells. Detailed analysis of the fatty acid patterns allowed various grouping of the species, whereas distinction between farmed and wild caught fish (and shrimp) seems to be the most obvious one. Characteristics for farmed fish are high proportions C18-unsaturated fatty acids (30 to 70 %), that occur due to high percentages of oelic acid (C18:1ω9), linoleic acid (C18:2ω6) and α-linolenic acid (C18:3ω3) as well as low ratios (approx. 1) of ω3 to ω6 fatty acids. Unsaturated fatty acids with longer chain length (≥ C20; LC-PUFA), that includes EPA and DHA were found in small (approx. 10 % for rainbow trout and atlantic salmon) to medium amounts (approx. 25 % for shrimp), which however, corresponds to high absolute values of EPA and DHA per 100 mg fish for species with high total lipid contents.

In contrast, wild caught species show low proportions of mentioned C18-fatty acids (10 to 30 %), but had high percentages of LC-PUFA. Especially for those species (e.g. Alaska pollock), who have low total lipid contents, values up to 60 % were observed, whereby more than 50 % are EPA and DHA. An increase of the percentages of LC-PUFA was observed for decreasing total lipid contents across all species. A value of 10 % seems to be the minimum limit for the proportions of LC-PUFA with increasing lipid content (Fig. 1; blue points). The omega-3-index (sum of percentages of EPA and DHA), that can be used for medicinal purposes is above 5 % for all species of the comparison group (two exceptions).

Fatty acid patterns of the samples from cell culture show major differences to those of the comparison group. Four derivatives (C16:0; C16:1ω7; C18:0 und C18:1ω9) are account for nearly 80 % of all fatty acids, whereby oelic acid (C18:1ω9) had by far the biggest proportions (48 to 58 %). LC-PUFA were also detected in the cultured cells, but with values up to 10 % they clearly below the species of the comparison group, that had similar total lipid contents (Fig. 1; orange points). Some fish samples had comparable percentages of LC-PUFA, but due to their higher lipid content, those samples had much higher absolute values.

Interestingly, the most prominent fatty acids EPA and DHA could be partly observed in larger proportions in the cells.

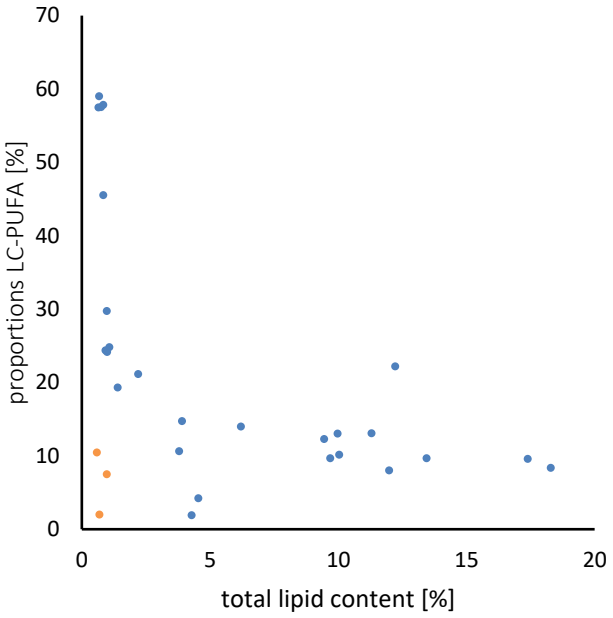

Figure 1.: Proportions of long-chain poly-unsaturated fatty acids (LC-PUFA) as function of the total lipid content. Fish of the comparison group: blue; cell culture: orange.

Conclusion

Fish cells, that were analysed within the CLEAN FISH project show many differences compared to traditional fish used as food, especially related to their fatty acid compositions. Therefore, it is still some research needed to align them closer to their natural role model. However, first promising indicators for a successful development are for example the omega-3-index or the ω3 to ω6-ratio up to 1.2 found in the cells from the lab of the current project.

Further information

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