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Determination of fish oil quality by ^{1}H NMR spectroscopy and multivariate statistics

1 Aim

Fish oil dietary supplements are extremely prone to oxidation because of their high contents of n-3 fatty acids. Common measures of fat quality are peroxide value (PV), anisidine value (AnV), and acid value (AV). However, the analysis of these parameters by traditional wet chemistry methods is time-consuming, work- and solvent-intensive and requires high amounts of sample. Therefore, in this study, ^{1}H NMR spectra and multivariate statistics (PLS regression and artificial neural networks (ANN)) were used to model PV, AnV, AV as well as the content of total n-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), the two main n-3 fatty acids in fish oil.

2 Materials and Methods

Materials

84 fish oils of different fish species, refined and unrefined, some of wich were stored under various conditions (varying light and temperature exposure) in order to increase the range of calibration

Methods

Analysis of PV, AnV and AV by wet chemistry

- PV according to Wheeler (DGF[a] C-VI 6a – part 1 (05)) with visual endpoint determination
- AnV according to DGF C-VI 6e (12)
- AV according to DIN EN ISO 660 with visual endpoint determination

Determination of fatty acid profile by GC-FID

- chromatographic analysis according to DGF C-VI 10a (00) after alkaline transesterification (DGF C-VI 11d(98))

^{1}H NMR spectroscopy

140 ± 1 mg oil were dissolved in 700 µL chloroform-d1 (0.03 % TMS) and analyzed by ^{1}H NMR spectroscopy (Bruker Avance III-HD 400 MHz) in two 1D experiments:

1) spectral width 8223.7 Hz, relaxation delay 4 s, no. of scans 16, acquisition time 3.9846 s, pulse width 90 °, pulse sequence zg, temperature 300 K
2) suppression of lipid signals and ^{13}C decoupling: spectral width 8223.7 Hz, relaxation delay 4 s, no. of scans 32, acquisition time 1.9923 s, pulse width 90 °, pulse sequence noesygpigps1d.comp2, temperature 300 K

The spectra were baseline-corrected and binned (interval width 0.002 ppm) in MestReNova, version 10.0. The regions containing the chloroform and the TMS signal were cut out of all spectra. Additionally, in case of the spectra of the second NMR experiment, the regions of signal suppression (0.84–2.90 ppm, 5.22–5.50 ppm) as well as the region containing hydroperoxide signals (8.20–8.60 ppm) were removed.

Statistical analysis

The spectra were further processed by mean centering and logarithmization in MATLAB, version 9.0. Variable selection and dimensionality reduction was also performed in MATLAB and comprised Monte Carlo-Uninformative Variable Elimination[b] (MC-UVE), Successive Projections Algorithm[c] (SPA) and PLS regression. The reduced data was used as input for artificial neural networks in MemBrain, version 06.01.02.00.

[a] German Society for Fat Science
[b] Li, H.D., Xu, Q.S., Liang, Y.Z. 2014. PeerJ PrePrints 2:e190v1, source codes available at www.libpls.net
[c] Araújo, M.C.U., Saldanha, T.C.B., Galvão, R.K.H., Yoneyama, T., Chame, H.C., Visani, V. 2001. Chemometrics and Intelligent Laboratory Systems. 57: 65–73.

3 Model characteristics

Table 1: Summary of performance characteristics for ANN and PLS regression models

			ANN				PLS[e]			
	n (total)	range	calibration RMSEC	validation[d] RMSEP	validation[d] Q^2	model characteristics	calibration RMSEC	validation[d] RMSEP	validation[d] Q^2	model characteristics
PV [meq/kg]	242	0.00-35.5	0.17	0.28	0.9985	PLS-ANN with mean-centered and logarithmized data: 20 input neurons, 20 hidden neurons, 1 hidden layer - NMR spectra 2)	0.0004	1.39	0.9611	mean-centered and logarithmized data, 34 components
AnV	239	0.00-113.1	0.72	0.74	0.9985	MC-UVE-SPA-ANN: 39 input neurons, 39 hidden neurons, 1 hidden layer - NMR spectra 2)	0.74	3.26	0.9712	mean-centered data, 40 components
AV [mg NaOH/g]	225	0.04-15.5	0.16	0.17	0.9962	PLS-ANN with mean-centered and logarithmized data: 15 input neurons, 1st hidden layer: 18 neurons, 2nd hidden layer: 1 neuron - NMR spectra 1)	0.12	0.92	0.8913	mean-centered and logarithmized data, 14 components
DHA [g/100g]	125	2.0-24.6	0.33	0.33	0.9853	PLS-ANN with mean-centered and logarithmized data: 15 input neurons, 29 hidden neurons, 1 hidden layer - NMR spectra 1)	0.03	1.06	0.8439	mean-centered and logarithmized data, 20 components
EPA [g/100g]	125	1.6-28.9	0.17	0.42	0.9955	PLS-ANN with mean-centered and logarithmized data: 15 input neurons, 15 hidden neurons, 1 hidden layer - NMR spectra 1)	0.63	1.65	0.9282	mean-centered and logarithmized data, 10 components
n-3 FA [g/100g]	125	10.5-57.2	0.20	0.27	0.9974	PLS-ANN with mean-centered and logarithmized data: 19 input neurons, 19 hidden neurons, 1 hidden layer - NMR spectra 1)	1.72	2.36	0.8044	mean-centered and logarithmized data, 8 components

[d] external validation with 15 % of samples
[e] based on the region -1.0-10.5 ppm

Q^2: Predictive coefficient of determination **RMSEC**: Root Mean Square Error of Calibration
RMSEP: Root Mean Square Error of Prediction

4 Goodness of prediction

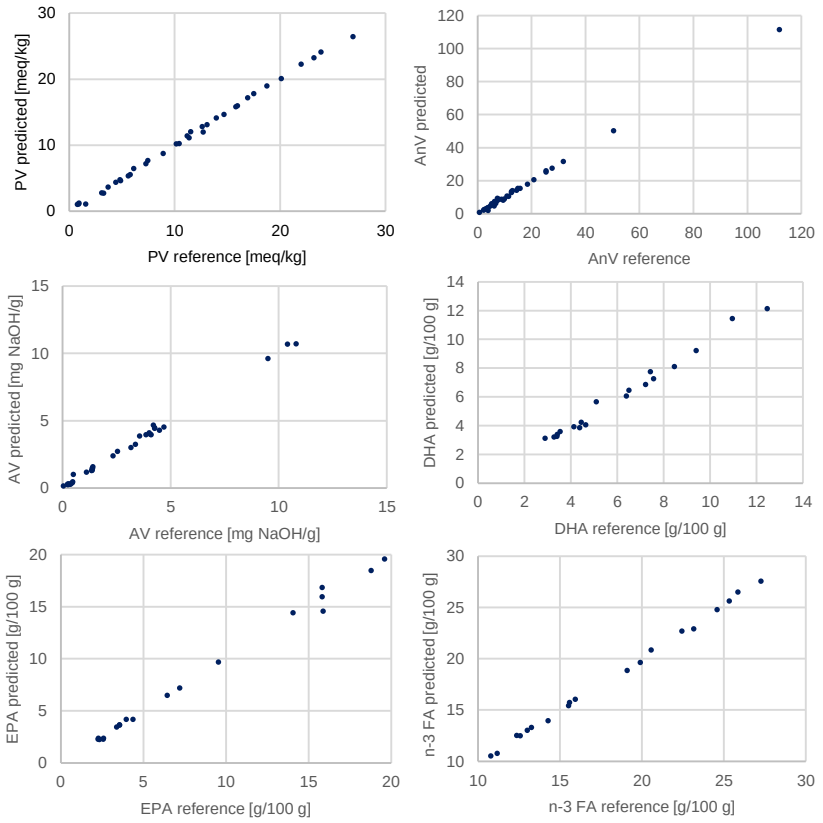

Fig. 1: Results of external validation for ANN models

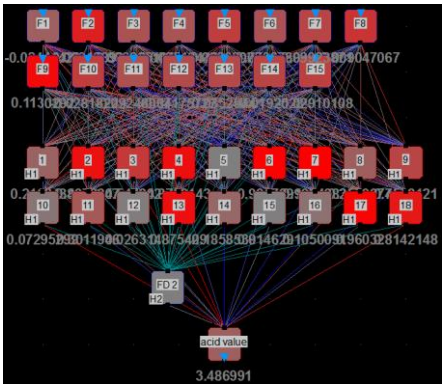

Fig. 2: Network architecture for AV ANN model

5 Conclusion

Important quality parameters of fish oil have been successfully modeled by ANN based on ^{1}H NMR spectra. The performance of the developed algorithms is superior to that of PLS regression models. However, PLS regression is useful as a preprocessing tool for ANN in reducing the dimensionality of the data. To the best of our knowledge, this is the first time that ANN are used in combination with NMR spectroscopy to predict the quality parameters analyzed in this study. Consequently, ^{1}H NMR spectroscopy in combination with multivariate statistics (ANN) can be considered a valuable tool for the quality assessment of fish oils.

Acknowledgement
The authors would like to thank Lipromar GmbH and Eurofins GfA Lab Service GmbH for providing the fish oil samples.