

Pollock Conservation Cooperative Research Center

PROGRESS REPORT

Project Title: "Purification of pollock oil using short path distillation"

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PROJECT ABSTRACT

The beneficial health effects of a diet rich in long chain polyunsaturated omega-3 fatty acids (LC- ω 3-PUFAs) have been fully described in recent years. Marine oils are an important dietary source of LC- ω 3-PUFAs, being especially rich on two of the most important fatty acids of this class namely, EPA (eicosapentaenoic acid; 20:5 ω 3) and DHA (docosahexaenoic acid; 22:6 ω 3). Due to its nutritional value, there is growing interest in refining fish oil for human consumption. In Alaska there are large quantities of fishery byproducts being used for the production of fish meal and fish oil. Most fish oil produced in Alaska is crude or unrefined, thus it can only serve as an ingredient for animal feed, unless further processing steps are included to handle fishery byproduct components as raw materials for the production of food. In this case, unrefined human-grade fish oil can be produced and may be further purified to meet specifications. The aim of this research is to investigate the applicability of short-path distillation for the purification of commercial pollock oil and human-grade pollock liver oil. Pollock oils will be subjected to short-path distillation for deodorization and removal of free fatty acids. The main advantages of using this technology, as compared to traditional fish oil purification steps, are that it reduces the use of chemicals during processing and decreases the number of steps needed to refine fish oils.

PROJECT OBJECTIVES

This research has three main objectives:

1. Determine and compare the chemical characteristics of crude and refined oils extracted from pollock livers from fish caught in waters surrounding Kodiak Island during early spring months (Feb.-Mar.; Pollock A season), to early fall months (Sept.-Oct.; Pollock B season).
2. Investigate the applicability of short-path distillation for the purification of crude pollock oil extracted from pollock livers from fish caught in waters surrounding Kodiak Island during early spring months (Feb.-Mar.; Pollock A season), and early fall months (Sept.-Oct.; Pollock B season).

3. Investigate the applicability of short-path distillation for the purification of crude pollock oil commercially produced during fall and spring months in Kodiak. In objectives 2 and 3 fish oil samples will be purified using the traditional fish oil purification steps previously described, as well as the alternative short-path distillation method. The chemical characteristics of purified pollock oil samples will be determined and compared.

PROJECT UPDATE

There were unexpected changes in the personnel available to work on this research project. Laboratory technician Mr. Steve Coen, listed in the budget for 1 month of salary, requested extended family leave. Mr. Coen has been on family leave since Oct. 08. Dr. Demir, listed in the budget for 7 months of salary, took responsibility for another research project at the University of Alaska Fairbanks, Fishery Industrial Technology Center (FITC) and could not participate in this research project. Thus, Visiting Research Scholar Ms. Vanessa Riberio from the Graduate Program of Food Engineering at FURG (Fundacao Universidade do Rio Grande, Brazil) was recruited. Ms. Ribeiro started conducting the project under Dr. Oliveira's guidance in first week of Sept 08; she will continue to develop the proposed research at FITC until Aug 09.

During the research proposal review process it was suggested that it would be best to conduct the research in pollock livers and oils from the pollock Bering Sea fisheries. Therefore, in the first week of September Mr. Richard Draves (VP Product Development, American Seafoods) and Dr. Oliveira traveled to Dutch Harbor to organize the collection of samples for B Season sampling period. The fish meal and fish oil operations of two American Seafoods catcher processor vessels were visited (Triumph and Northern Jaeger) and it was decided that the catcher-processor 'Triumph' could produce a trial run of pollock liver oil. Oil was rendered onboard by passing ground fresh livers through a sequence of three inline horizontal cookers operated at about 85-90C. The material was then separated into oil, water, and a protein sludge using a three-phase centrifuge. Plain liver oil and liver oil with additive (ascorbyl palmitate) produced by the 'Triumph' crew were shipped to Kodiak in Oct. of 08. Catcher-processor 'Northern Eagle' (American Seafoods) shipped pollock oil samples produced from a mix of byproducts to Kodiak in Oct. 08. Pollock oil samples containing the additive ascorbyl palmitate were also produced on board of the 'Northern Eagle' and shipped to Kodiak in Oct. 08. Hake oil was also included in this study as per the request of American Seafoods; this oil arrived in Kodiak during Nov. 08 for chemical characterization. Two independent samples of each of the five oils (liver oil, liver oil with additive, pollock oil, Pollock oil with additive, and hake oil) were chemically characterized using methods described in the proposal. Results were delivered to American Seafoods in Dec. 08. Environmental contaminants and fat soluble vitamins analyses were added to the scope of the research as part of the chemical characterization of these oils. The chemical characteristics of these five oils are presented in this report.

The crew of the catcher-processor 'Triumph' collected pollock livers during B Season. Livers were frozen in eighteen blocks of 7.5 Kg each and samples shipped to Kodiak in Oct. 08. After livers were thawed, ten 0.5 Kg liver homogenate batches were produced

and proximate composition (protein, lipid, moisture and ash content) was determined. Oils were then rendered from liver homogenate samples at a lower temperature than the planned 80 °C. This change was needed to verify if high temperature rendering of livers onboard (85-90°C; ~2 min.) would yield oil differ in composition from pollock livers produced under laboratory conditions at low temperature (60 °C and 50 °C; 30 min.). Water was not added to the livers as proposed, and a high-speed centrifuge (7,500 rpm for 30 min) was used instead of filtration to separate oil, water, and protein. These adaptations in the procedure to render liver oil in the laboratory were needed to bring the method closer to the onboard processing. Due to intrinsic variability in raw material composition, the number of replicates per extraction for processing was increased from three to five. Ten oil samples were produced for B season livers and chemically characterized. As planned, the protein fractions recovered from the rendering of oil were freeze-dried and shipped to Dr. Peter Bechtel (ARS/USDA) in Fairbanks for chemical characterization.

Sampling of A season pollock livers and pollock oil from whole byproduct will take place from late Feb.-early Mar. During this season it is not possible to produce liver oil onboard because livers are separated from viscera during B season using the pollock roe sorting area. Only five liver oil batches will be produced for livers collected during A season. Liver oils will be produced from A Season livers only at 60 °C because this temperature provided higher processing yields than rendering at 50 °C.

The short-path distillation unit was purchased in early Aug. 08 and arrived at FITC (Kodiak) during the last week of Sept. The unit was assembled in October, but preliminary tests and purification of oils have been delayed.

The timeline has been revised to reflect the increase in number of samples investigated for the B Season sampling period, and to reflect the delays caused by unexpected changes that occurred in personnel. The project start date was subject to a 2-month delay, and the projection is that the project will take 12 instead of 9 months to be completed. The end date of the project, as shown in the revised timeline below, has been updated to reflect these changes.

REVISED TIMELINE

Tasks	July-Sept. 2008	Oct-Dec 2008	Jan-Mar 2009	Mar-Aug 09
Setup and optimization of Short-path distillation unit methodology using test oils	X (DELAYED)		X (STARTING IN FEB 09)	
Sampling	X (SAMPLED SEPT AND OCT)	X (SAMPLES ARRIVED FITC)	X (STARTING AS PLANNED)	
Liver oil extraction and stabilization	X (AS PLANNED)		X (STARTING AS PLANNED)	

Analysis of stabilized crude oil samples	X (AS PLANNED)	X (AS PLANNED)	X (CONTINUING AS PLANNED)	
Purification of fish oils		X (DELAYED)	X (STARTING IN MAR 09)	X (CONTINUING THROUGH JUN 09)
Analysis of refined oil samples			X (DELAYED)	X (STARTING APR 09)
Data analysis and preparation of deliverables		X (AS PLANNED)	X (CONTINUING AS PLANNED)	X (CONTINUING THROUGH AUG 09)

RESULTS

Chemical composition of commercial oils sampled during B Season

The following collection information was available for each commercial oil sample:

- 'Normal' pollock oil, Northern Eagle, 11/10/08. Sample code: PO
- 'Normal' pollock oil with ascorbyl palmitate, Northern Eagle, 11/10/08. Sample code: POAP
- Liver oil, Triumph, 10/22/08. Sample code: LO
- Liver oil with ascorbyl palmitate (AP), Northern Eagle, 10/22/08. Sample code LOAP
- Hake oil received late Nov 08.

Analyses of lipid classes were performed in triplicate for each of the oils. Table 1 shows the results for this analysis. Oils are comprised mostly of triacylglycerides presenting only traces of free fatty acids and phospholipids. Modest amounts of sterols were observed and are in the range for other fish oils but lower than most crude Pollock oils previously investigated that were rendered from byproducts.

Table 1. Lipid classes analysis of commercial oils

	TG	FFA	DG/ST	MG	PL
Normal Pollock oil	98.2	1.2	0.6	0	0
Normal Pollock oil w/ AP	99.4	0.00	0.6	0	0
Liver oil	99.3	0.2	0.3	0	0.1
Liver oil w/ AP	100.0	0.00	0.00	0	0
Hake	99.5	0	0.5	0	0

TG triacylglycerides; FFA free fatty acids; DG/ST diacylglycerols/sterols (co-eluting classes); MG monoacylglycerides; PL phospholipids

Color of oils was determined using the L*a*b* scale with the CR-300 Chroma Meter (Minolta, Japan). The color parameters measured are lightness (L*), chromaticity of red/green (a*), and chromaticity of blue and yellow (b*). These parameters are in accordance with the recommendations of the International Commission on Illumination, (CIE). The Minolta Chroma Meter measures the reflectance of light from a sample surface compared to a white standard calibration plate using illuminant D65 as the source of light. Color analysis revealed large differences in color between oil samples. Two commercial oils were used for comparison:

- Commercial 18-month old human-grade salmon oil stored at room temperature and without precautions to reduce oxidation, this oil was purposely left to oxidize.
- Commercial feed grade herring oil stored under chilled conditions and purchased during the fall of 2008.

Normal pollock oil was turbid and seems to have a layer of water forming at the bottom of the containers (under investigation), low L values reflect the turbidity of the sample. Turbidity was not observed in the normal pollock oil with additive. Liver oil and liver oil with additive were more yellow than normal pollock oil, which was reddish orange. Bering Sea pollock is feeding heavily on krill during summer and fall and diet is the main cause of the orange/reddish color observed for pollock oils produced from a mixture of pollock byproducts. Note that salmon oil is also reddish-orange while herring oil is yellowish as the Pollock liver oils.

Table 2. L*a*b* values (color) and indices of oxidative stability of commercial oils

	% FFA	PV(meq/Kg)	Color		
			L*	a*	b*
PO	3.6	7.8	30.0	9.2	45.5
POAP	3.5	7.6	45.4	13.6	72.2
LO	3.2	5.7	47.3	-5.6	25.2
LOAP	2.1	4.7	51.3	-5.7	27.5
Herring oil	4.1	8.6	52.3	-6.2	48.0
Salmon Oil	13.3	49.9	54.4	5.0	60.0

PO normal pollock oil; LO pollock liver oil; AP ascorbyl palmitate



Figure 1. Picture of commercial oils from left to right LO, LOAP, POAP, PO, herring

The free fatty acid (FFA) values show that all pollock oils and liver oils had lower %FFA than either herring or salmon oil; however, all levels are above the desirable specification for edible oils (0.1-0.3%). Peroxide values (PV) measure primary products of lipid oxidation. The desirable level for edible oils is in the range of 1-3 meq./Kg., and salmon oil value indicates severe oxidation (fish odors present). Even though PV values of 4-8 do not indicate severe oxidation, these values are higher than the target for edible oils. A storage stability study should be conducted to observe the effect of ascorbyl palmitate over time under low and ambient storage conditions.

Fatty acid profiles were determined for hake oil samples and are presented in Table 3. The fatty acid profile of hake was typical of cold water fish species. Note that about 25% of the oil is comprised of omega-3 FA with DHA and EPA making up approximately 9% and 13% of the oil. Data in mg/g of oil showed an average of 74 mg/g oil of DHA and about 104 mg/g oil of EPA. Other abundant FA were 16:0 (palmitic acid), 16:1 ω 7 (palmitoleic acid), and 18:1 ω 9 (*cis*-oleic acid). Fatty acid profiles were determined for normal pollock oil with and without AP and Pollock liver oil with and without AP are shown in Table 4 together with the profile of commercial herring oil (feed grade). Table 5 is similar to Table 4 but the results are reported in mg per g oil. As expected, little differences were observed in the profiles of the oils since most of the lipids from pollock byproducts are present in the liver tissue, especially during B season byproducts when livers have the highest lipid content.

Table 6 and 7 report the results of environmental contaminants and fat soluble vitamins present in the commercial oils. Herring oil results are provided for comparison. Environmental contaminants analysis was performed by MVTL Laboratories (Minnesota) while fat soluble vitamins analysis was conducted by Warren Analytical laboratories (Colorado). Contaminants are not a concern in any of the oils investigated with levels of all quantified contaminants being very low. The fat soluble vitamins results were highly variables and difficult to interpret. Thus, samples of all six oils were shipped in duplicate to Dr. Peter Bechtel's laboratory and vitamins A, E and K will be determined using a LCMS system instead of a HPLC system coupled to a fluorescent detector.

Table 3. Fatty acids profile of two independent hake oil samples (A and B are replicates)

	Hake oil A	Hake oil B	Hake oil A	Hake oil B	Average	Average
	mg/g	mg/g	% w/w	% w/w	mg/g	% w/w
14:0	11.58	11.58	1.44	1.43	11.58	1.44
15:0	1.59	1.60	0.20	0.20	1.59	0.20
16:0	168.38	168.56	20.88	20.87	168.47	20.88
16:1 ω 11	1.70	1.71	0.21	0.21	1.71	0.21
16:1 ω 9	1.50	1.52	0.19	0.19	1.51	0.19
16:1 ω 7	48.34	48.29	5.99	5.98	48.31	5.99
16:1 ω 5	1.71	1.81	0.21	0.22	1.76	0.22
Iso 17:0	0.94	0.95	0.12	0.12	0.94	0.12
Ante iso 17:0	4.24	4.25	0.53	0.53	4.24	0.53
17:0	2.56	2.56	0.32	0.32	2.56	0.32
Unknown	10.15	10.10	1.26	1.25	10.13	1.25
17:1 ω 9	3.61	3.60	0.45	0.45	3.60	0.45
Iso or Anteiso 18:0	2.88	2.92	0.36	0.36	2.90	0.36
18:0	25.02	25.09	3.10	3.11	25.06	3.11
18:1 ω 9 <i>cis</i>	218.21	218.26	27.06	27.02	218.24	27.04
18:1 ω 7	48.32	48.36	5.99	5.99	48.34	5.99
18:1 ω 5	1.64	1.61	0.20	0.20	1.63	0.20
18:2 ω 6 <i>cis</i>	7.95	7.89	0.99	0.98	7.92	0.98
18:3 ω 6	2.25	2.21	0.28	0.27	2.23	0.28
18:3 ω 3	4.89	4.84	0.61	0.60	4.86	0.60
18:4 ω 3	10.18	10.36	1.26	1.28	10.27	1.27
Iso or Ante iso 20:0	1.29	1.32	0.16	0.16	1.30	0.16
20:1 ω 11	5.67	5.64	0.70	0.70	5.65	0.70
20:1 ω 9	10.93	10.91	1.36	1.35	10.92	1.35
20:1 ω 7	2.08	2.09	0.26	0.26	2.09	0.26
20:2 ω 6	1.54	1.54	0.19	0.19	1.54	0.19
20:4 ω 6	5.93	5.85	0.74	0.72	5.89	0.73
20:4 ω 3	3.55	3.58	0.44	0.44	3.56	0.44
20:5 ω 3 (EPA)	104.01	104.54	12.90	12.94	104.28	12.92
22:1 ω 11	5.94	5.94	0.74	0.74	5.94	0.74

22:1 ω 9	2.27	2.22	0.28	0.27	2.24	0.28
22:2 ω 6	4.59	4.59	0.57	0.57	4.59	0.57
22:5 ω 3	7.26	7.21	0.90	0.89	7.24	0.90
22:6 ω 3 (DHA)	73.72	74.15	9.14	9.18	73.94	9.16
Saponifiables	806.40	807.65	100.00	100.00	807.03	100.00
Σ SAT	224.15	224.46	27.80	27.79	224.30	27.79
Σ MUFA	348.31	348.36	43.19	43.13	348.33	43.16
Σ PUFA	225.85	226.77	28.01	28.08	226.31	28.04
Σ ω-3	203.59	204.69	25.25	25.34	204.14	25.30

SAT saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids A and B are replicates

Table 4. FA profiles of pollock oils in %w/w

	PO A	PO B	LO A	LO B	POAP A	POAP B	LOAP A	LOAP B	Herring oil A	Herring oil B
14:0	5.28	5.34	5.39	5.42	5.44	5.38	5.44	5.38	4.75	4.75
14:1ω 5	0.12	0.12	0.00	0.00	0.12	0.12	0.13	0.13	0.09	0.09
15:0	0.19	0.19	0.19	0.20	0.19	0.19	0.19	0.19	0.30	0.30
16:0	8.83	8.87	9.79	9.80	8.85	8.82	10.08	9.98	10.14	10.13
16:1ω 11	0.20	0.20	0.17	0.17	0.20	0.20	0.17	0.17	0.18	0.19
16:1ω 7	10.43	10.49	11.88	11.83	10.44	10.39	11.86	11.79	8.23	8.22
16:15	0.31	0.31	0.29	0.29	0.31	0.31	0.29	0.29	0.24	0.24
17:0	0.60	0.60	0.64	0.64	0.61	0.61	0.63	0.63	0.64	0.64
18:0	1.34	1.34	1.46	1.54	1.27	1.26	1.49	1.46	0.81	0.80
18:1ω 9 <i>trans</i>	2.14	2.30	1.99	2.01	2.03	2.07	1.98	1.97	0.63	0.63
18:1ω 9 <i>cis</i>	5.99	6.01	7.49	7.47	5.43	5.44	7.47	7.46	5.46	5.46
18:1ω 7	3.51	3.52	4.46	4.42	3.29	3.27	4.45	4.44	2.07	2.06
18:1ω 5	0.54	0.54	0.48	0.47	0.55	0.54	0.48	0.47	0.52	0.52
18:2ω 6 <i>cis</i>	0.58	0.58	0.52	0.53	0.57	0.57	0.51	0.51	0.87	0.87
18:3ω 6	0.35	0.34	0.37	0.38	0.36	0.36	0.36	0.36	0.00	0.00
18:3ω 3	0.32	0.32	0.25	0.26	0.32	0.31	0.24	0.24	0.50	0.51
18:4ω 3	1.61	1.61	1.27	1.24	1.62	1.62	1.25	1.23	1.39	1.40
20:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.20
20:1ω 11	15.39	15.44	14.45	14.32	15.58	15.54	14.33	14.35	1.62	1.56
20:1ω 9	4.46	4.48	3.96	3.95	4.48	4.46	3.94	3.94	15.83	15.87

20:1 ω 7	0.30	0.30	0.31	0.30	0.30	0.30	0.30	0.31	1.05	1.05
20:1 ω 5	0.12	0.00	0.00	0.00	0.13	0.13	0.00	0.00	0.00	0.00
20:2 ω 6	0.14	0.14	0.00	0.15	0.14	0.14	0.00	0.13	0.13	0.13
20:4 ω 3	0.38	0.39	0.33	0.33	0.38	0.38	0.33	0.32	0.29	0.29
20:5 ω 3	10.16	10.19	10.94	10.85	10.29	10.22	10.80	10.85	5.86	5.84
22:1 ω 11	14.62	14.72	12.71	12.63	15.25	15.20	12.65	12.65	27.35	27.34
22:1 ω 9	1.03	1.04	0.92	0.94	1.08	1.07	0.92	0.92	2.51	2.51
22:1 ω 7	0.30	0.30	0.28	0.27	0.33	0.33	0.27	0.27	0.46	0.46
22:2 ω 6	0.50	0.50	0.53	0.55	0.52	0.52	0.53	0.53	0.27	0.27
22:5 ω 3	0.81	0.80	0.73	0.71	0.80	0.81	0.73	0.73	0.76	0.76
22:6 ω 3	5.23	5.27	4.95	4.91	5.19	5.13	4.81	4.87	3.84	3.83
24:1 ω 9	0.44	0.43	0.34	0.36	0.47	0.45	0.36	0.34	0.60	0.58
Saponifiables	96.51	96.86	97.08	97.08	96.67	96.42	96.99	97.06	97.61	97.62
Σ SAT	16.23	16.34	17.47	17.60	16.36	16.26	17.83	17.64	16.84	16.82
Σ MUFA	60.04	60.20	59.73	59.42	59.98	59.96	59.61	59.51	66.86	66.78
Σ PUFA	20.23	20.31	19.89	20.05	20.32	20.20	19.56	19.91	13.92	14.02
$\Sigma \omega$ -3	18.50	18.58	18.47	18.30	18.59	18.47	18.16	18.25	12.65	12.62

SAT saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids

PO normal pollock oil; LO liver oil; AP ascorbyl palmitate; A and B are replicates

Table 5. FA profiles of commercial oils in mg/ g oil

	PO A	PO B	LO A	LO B	POAP A	POAP B	LOAP A	LOAP B	Herring oil A	Herring oil B
14:0	46.69	47.74	43.68	45.92	46.44	47.20	46.12	45.84	42.59	41.15
14:1 ω 5	1.08	1.09	0.00	0.00	1.03	1.04	1.14	1.13	0.82	0.78
15:0	1.64	1.67	1.53	1.72	1.62	1.65	1.60	1.59	2.66	2.56
16:0	78.08	79.24	79.34	82.95	75.56	77.32	85.55	85.03	90.91	87.78
16:1 ω 11	1.73	1.75	1.34	1.41	1.70	1.74	1.45	1.46	1.60	1.61
16:1 ω 7	92.19	93.75	96.23	100.14	89.19	91.08	100.65	100.49	73.72	71.23
16:1 ω 5	2.77	2.80	2.39	2.47	2.64	2.72	2.48	2.48	2.18	2.12
17:0	5.27	5.34	5.15	5.45	5.24	5.35	5.34	5.36	5.70	5.51
18:0	11.85	12.01	11.85	13.02	10.87	11.09	12.68	12.47	7.23	6.93
18:1 ω 9 <i>trans</i>	18.95	20.56	16.15	16.98	17.36	18.15	16.82	16.78	5.68	5.45
18:1 ω 9 <i>cis</i>	52.93	53.69	60.66	63.29	46.38	47.69	63.42	63.53	48.97	47.29

18:1 ω 7	31.00	31.45	36.13	37.46	28.13	28.71	37.73	37.85	18.57	17.87
18:1 ω 5	4.75	4.82	3.90	3.95	4.67	4.78	4.04	4.04	4.65	4.51
18:2 ω 6 <i>cis</i>	5.16	5.21	4.18	4.47	4.90	5.02	4.33	4.33	7.81	7.57
18:3 ω 6	3.11	3.06	2.98	3.22	3.04	3.12	3.08	3.09	0.00	0.00
18:3 ω 3	2.82	2.84	2.00	2.22	2.69	2.74	2.07	2.08	4.52	4.41
18:4 ω 3	14.21	14.35	10.30	10.51	13.85	14.17	10.60	10.49	12.49	12.11
20:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.82	1.73
20:1 ω 11	136.04	137.92	117.13	121.29	133.04	136.25	121.55	122.27	14.55	13.49
20:1 ω 9	39.42	40.00	32.10	33.41	38.22	39.11	33.39	33.55	141.92	137.46
20:1 ω 7	2.66	2.68	2.48	2.53	2.56	2.64	2.58	2.61	9.43	9.09
20:4 ω 3	3.36	3.48	2.65	2.79	3.24	3.36	2.80	2.76	2.60	2.51
20:5 ω 3	89.79	91.07	88.68	91.85	87.85	89.62	91.59	92.48	52.55	50.56
22:1 ω 11	129.24	131.51	102.99	106.97	130.24	133.25	107.29	107.75	245.14	236.84
22:1 ω 9	9.13	9.28	7.47	7.93	9.19	9.39	7.83	7.84	22.47	21.71
22:1 ω 7	2.64	2.68	2.25	2.30	2.85	2.91	2.32	2.32	4.14	4.01
22:2 ω 6	4.45	4.49	4.32	4.62	4.45	4.53	4.47	4.51	2.43	2.31
22:5 ω 3	7.13	7.19	5.88	6.00	6.85	7.08	6.22	6.18	6.79	6.56
22:6 ω 3	46.21	47.07	40.14	41.57	44.28	45.02	40.80	41.53	34.40	33.18
24:1 ω 9	3.93	3.82	2.76	3.07	3.97	3.99	3.05	2.93	5.33	5.06
Saponifiables	884.02	893.32	810.31	846.82	853.11	876.86	848.45	852.07	896.26	866.25
Σ SAT	143.52	146.00	141.54	149.06	139.73	142.61	151.28	150.29	150.91	145.67
Σ MUFA	530.78	537.80	483.98	503.22	512.29	525.76	505.72	507.04	599.20	578.52
Σ PUFA	178.85	181.43	161.13	169.80	173.57	177.13	165.95	169.66	124.77	121.41
Σ ω-3	163.52	165.99	149.66	154.93	158.77	161.98	154.08	155.52	113.35	109.33

SAT saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids

PO normal pollock oil; LO pollock liver oil; AP ascorbyl palmitate; A and B are replicates

Table 6. Contaminant analysis of commercial oils (ppm)

	PO	POAP	LO	POAP	Herring oil	Hake oil
Date Ext/Org-P						
Diazinon	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14	ND
Ethion	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14	ND
Malathion	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14	ND

Methyl Parathion	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14	ND
Parathion	< 0.12	< 0.12	< 0.12	< 0.12	< 0.12	ND
Ronnel	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13	ND
Carbophenothion (Trithion)	< 0.15	< 0.15	< 0.15	< 0.15	< 0.15	ND
Disulfoton	< 0.15	< 0.15	< 0.15	< 0.15	< 0.15	ND
Phorate (Thimet)	< 0.15	< 0.15	< 0.15	< 0.15	< 0.15	ND
Date Ext /SC PCB`s-Pest						
PCB-1016	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
PCB-1221	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
PCB-1232	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
PCB-1242	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
PCB-1248	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
PCB-1254	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
PCB-1260	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Aldrin	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Alpha-BHC	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
2,4`-DDD [O,P-DDD]	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
4,4`-DDD	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
2,4`-DDE [O,P-DDE]	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
4,4`-DDE	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
2,4`-DDT [O,P-DDT]	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
4,4`-DDT	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
Dieldrin	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Endrin	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Heptachlor	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Heptachlor Epoxide	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Hexachlorobenzene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Gamma-BHC(Lindane)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Methoxychlor	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Mirex	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Toxaphene	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Endosulfan (Thiodan)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Beta-BHC	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Delta-BHC	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Alpha Chlordane	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Gamma Chlordane	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

PO normal pollock oil; LO pollock liver oil; AP ascorbyl palmitate

Table 7. Fat soluble vitamins of commercial oils

	Vitamin A (IU/100g)	Vitamin E (IU/100g)	Vitamin D (IU/100g)	Vitamin K (µg/100g)
PO	94,309	13.0	3,126.0	<5.0
POAP	58,264	21.0	3,852.0	<5.0
LO	72,372	9.70	2,186.0	6.8
LOAP	81,994	13.2	1,962.0	6.0
Herring oil	45,071	5.68	1,377.0	<5.0
Hake oil	487,674	25.20	1,836.8	13.7

PO normal pollock oil; LO pollock liver oil; AP ascorbyl palmitate

The rendering of liver oils and their chemical composition

The rendering of oils was performed as five independent replicate extractions and following the procedure depicted below (Figure 2). Each batch of livers (2.5 Kg) was homogenized with a commercial blender for 2 minutes at 21.000 rpm. Proximate composition of homogenized tissue was in average 11.8% protein, 49.8% lipids, 0.3% ash and 38.1% moisture. Lipids were preserved in amber vials suspended in isooctane with 0.01% BH. Homogenized tissues were stored in amber glass vials under nitrogen and frozen at -35°C for less then 30 days until oil extraction. Liver homogenates were heated in a silicon bath to either 50 °C or 60 °C for 30 min with constant agitation as shown in Figure 3.

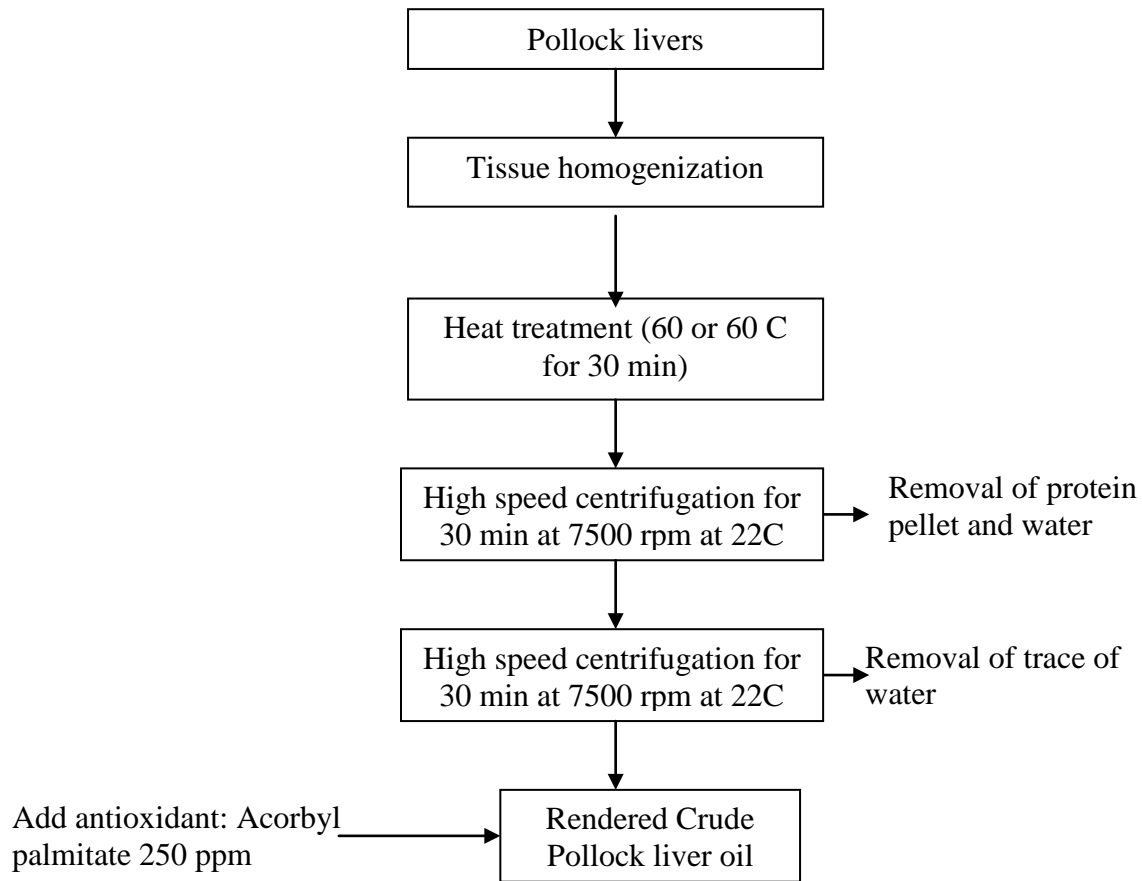


Figure 2. Schematics of the laboratory procedure to render oil from pollock livers



Figure 3. Picture of the laboratory apparatus to render oil from pollock liver

The protein pellet separated after the first centrifugation step was freeze-dried using the processing parameters described in Table 8 to produce pollock liver protein powders. Processing parameters and yields are shown in Tables 9 for liver oils rendered at 60C and in Table 10 for liver oils rendered at 50C. No significant difference in yields was recorded for liver oils rendered at higher temperature.

Table 8. Freeze drying steps used for pollock liver protein powders

Steps	Temperature (C)	Time (min)
01	-20	1000
02	0	1000
03	+10	1000
04	+25	1000

Table 9. Processing parameters and yields for liver oils rendered at 60C

	Ext 1	Ext 2	Ext 3	Ext 4	Ext5	Average
Sample weight (g)	502.3	501.7	502.3	502.3	506.1	502.9
Weight of oil after 1 st centrifugation (g)	290.8	290.1	285.0	290.4	289.2	289.1
Weight of oil after 2 nd centrifugation (g)	260.6	270.0	261.0	260.1	263.6	263.1
Processing yield (%)	51.9	53.8	52.0	51.8	52.1	52.3

Ext extraction replicates

Table 10. Processing parameters and yields for liver oils rendered at 50C

	Ext 6	Ext 7	Ext 8	Ext 9	Ext10	Average
Sample weight (g)	501.3	506.1	500.4	501.5	513.2	504.5
Weight of oil after 1 st centrifugation (g)	280.6	306.5	300.7	289.8	289.5	293.4
Weight of oil after 2 nd centrifugation (g)	251.4	261.3	261.2	251.3	263.7	257.8
Processing yield (%)	50.1	51.6	52.2	50.1	51.4	51.1

Ext extraction replicates

Protein powders were vacuum packaged and frozen at -80C until analysis. Samples of protein powders were provided to Dr. Peter Bechtel for characterization of amino acids and minerals. The proximate composition of the protein powders in dry basis is depicted in Figure 4.

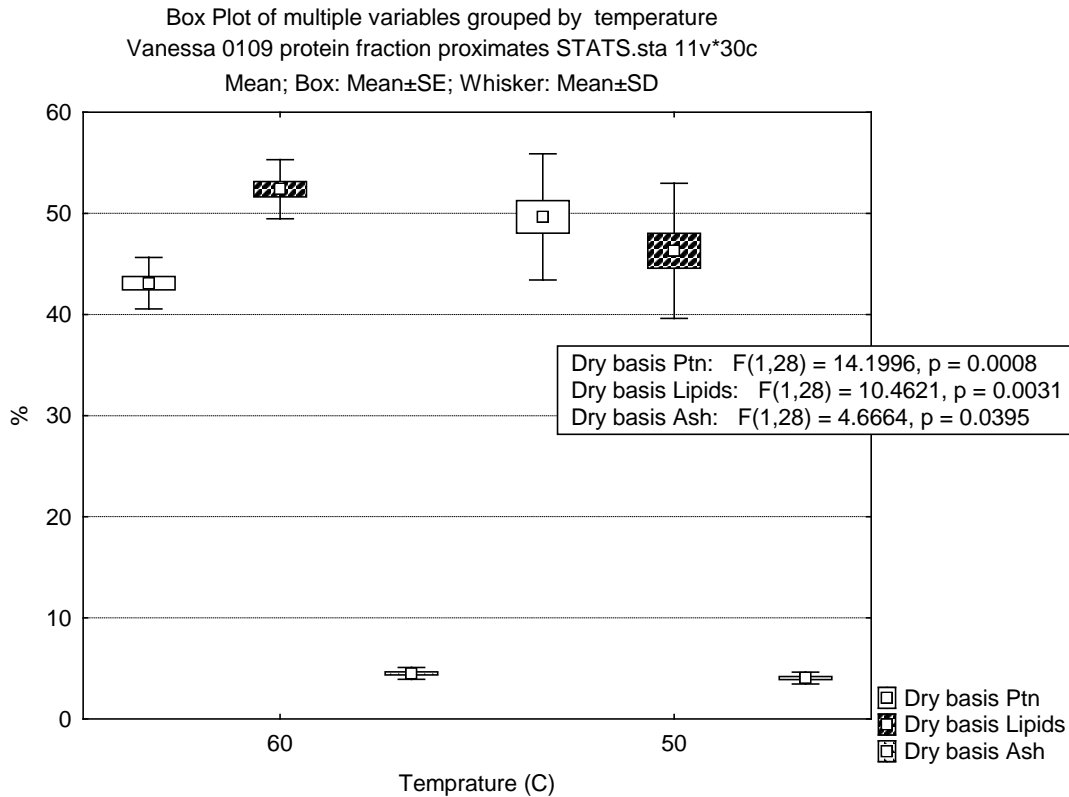


Figure 4. Proximate composition of Pollock liver protein powders

The average values determined in the color analysis are shown in Figure 5, where they can be easily compared with the color analysis of the commercial oils. Figures 6 and 7 depict color of liver oils rendered in our laboratory at 50 °C and 60 °C, respectively. Liver oils tend to be lighter in color and more yellow while pollock oil produced from a mixture of byproducts during B season is darker and red. The average values of water activity (a_w) of the rendered oils is shown in Figure 8 together with the a_w results of the commercial oils. Commercial pollock oil has significantly higher water activity than liver oils, and this may help explain the turbidity of pollock oil samples. Figures 9, 10 and 11 show average free fatty acids (%FFA), peroxide values (PV) and anisidine values (AV) for the liver oils rendered in the laboratory and the commercial oils. The %FFA values are higher in oils produced in the laboratory, and this reflects enzymatic activity that occurred during storage of the livers in frozen blocks. Enzymes are not completely inactive in this tissue even at -30C storage. Note that oils produced onboard derived from the processing of very fresh byproducts. The PV values reflect levels of primary products of lipid oxidation and the oils produced in the laboratory have higher PV because of the longer heating time during processing when compared to the very short time livers are subjected to heating during onboard processing (~2 min.). The higher levels of AV for

commercial liver oils are surprising since AV reflects the abundance of the secondary products of lipid oxidation. More information about secondary products of oxidation in oil samples will be available after we determine TBARS values. Finally, the average fatty acids composition of the liver oils produced in our laboratory is shown in Table 13. These results are similar to the fatty acids profiles determined for the commercial oils and other pollock oils previously investigated.

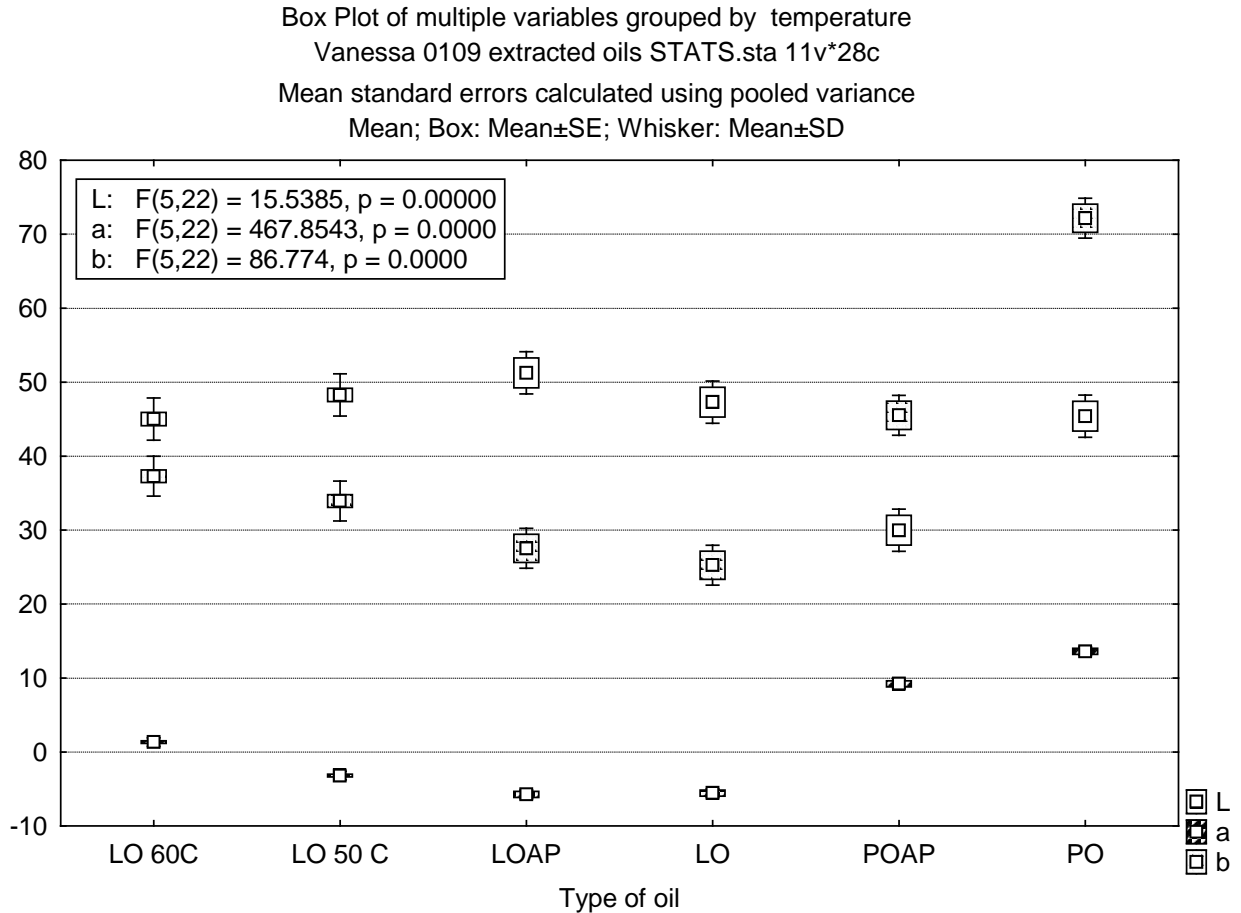


Figure 5. Color analysis of liver oils rendered in the laboratory and commercial oils.
LO 50C and 60C denote liver oils rendered in the laboratory at given temperatures. PO and LO denote pollock oil and pollock liver oil produced commercially by American Seafoods. AP ascorbyl palmitate.



Figure 6. Picture of LO produced at 50C



Figure 7. Picture of LO produced at 60C

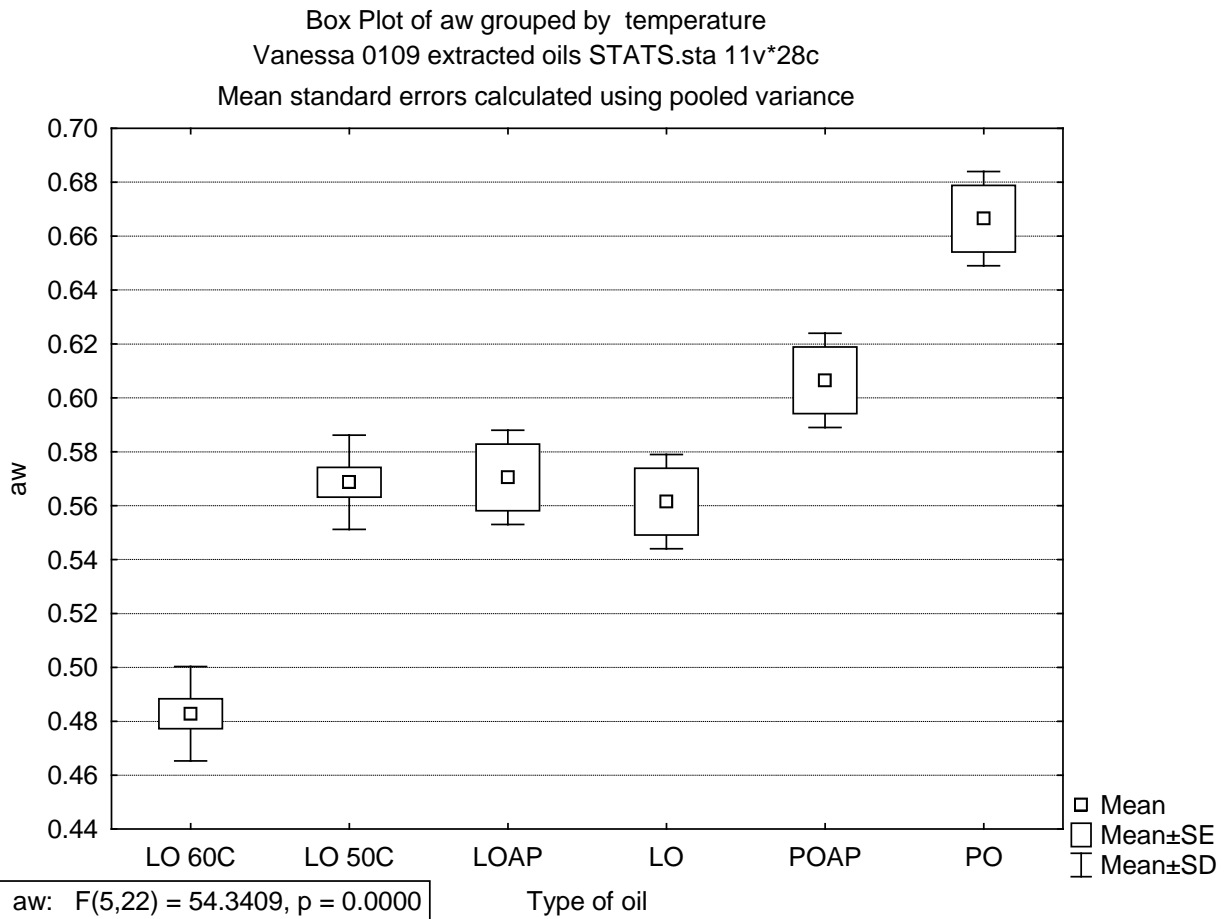
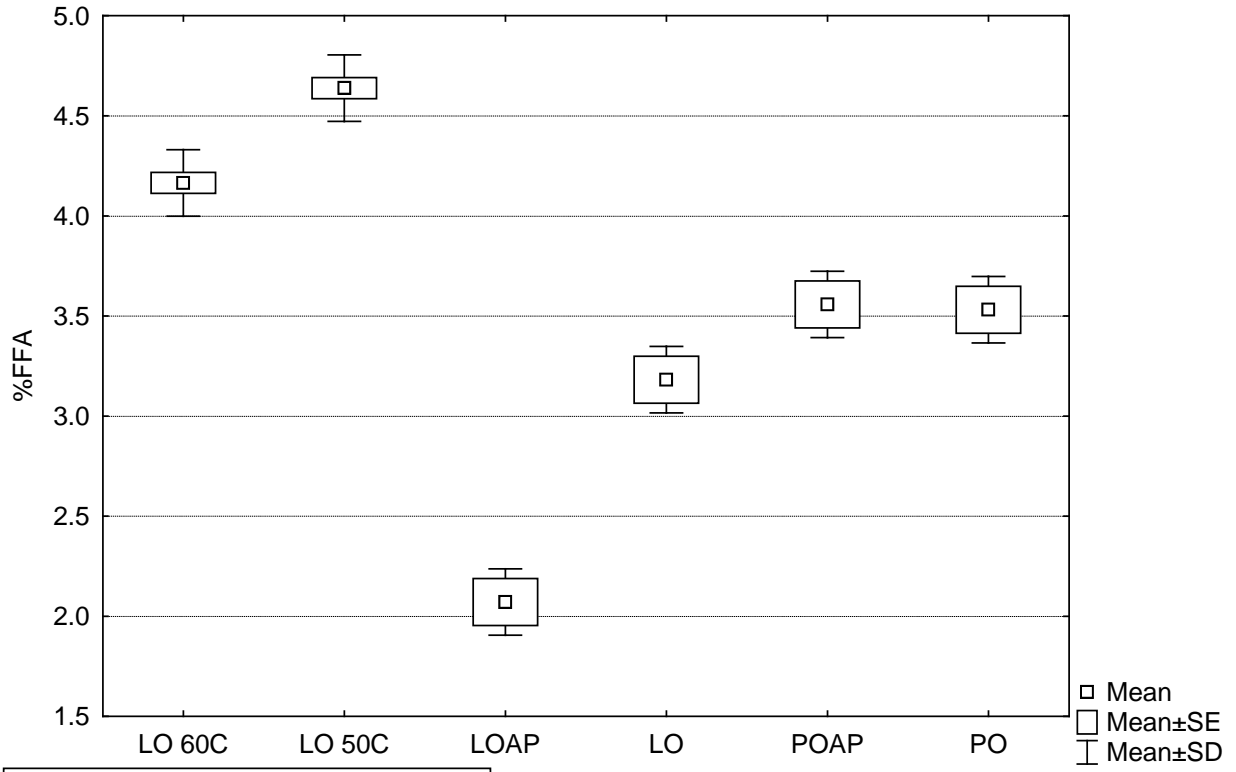


Figure 8. Water activity of liver oils rendered in the laboratory and commercial oils. LO 50C and 60C denote liver oils rendered in the laboratory at given temperatures. PO and LO denote pollock oil and pollock liver oil produced commercially by American Seafoods. AP ascorbyl palmitate.

Box Plot of %FFA grouped by temperature
 Vanessa 0109 extracted oils STATS.sta 11v*28c
 Mean standard errors calculated using pooled variance



%FFA: $F(5,22) = 101.1826, p = 0.0000$ Type of oil

Figure 9. Free fatty acids content (%) in liver oils rendered in the laboratory and commercial oils. LO 50C and 60C denote liver oils rendered in the laboratory at given temperatures. PO and LO denote pollock oil and pollock liver oil produced commercially by American Seafoods. AP ascorbyl palmitate.

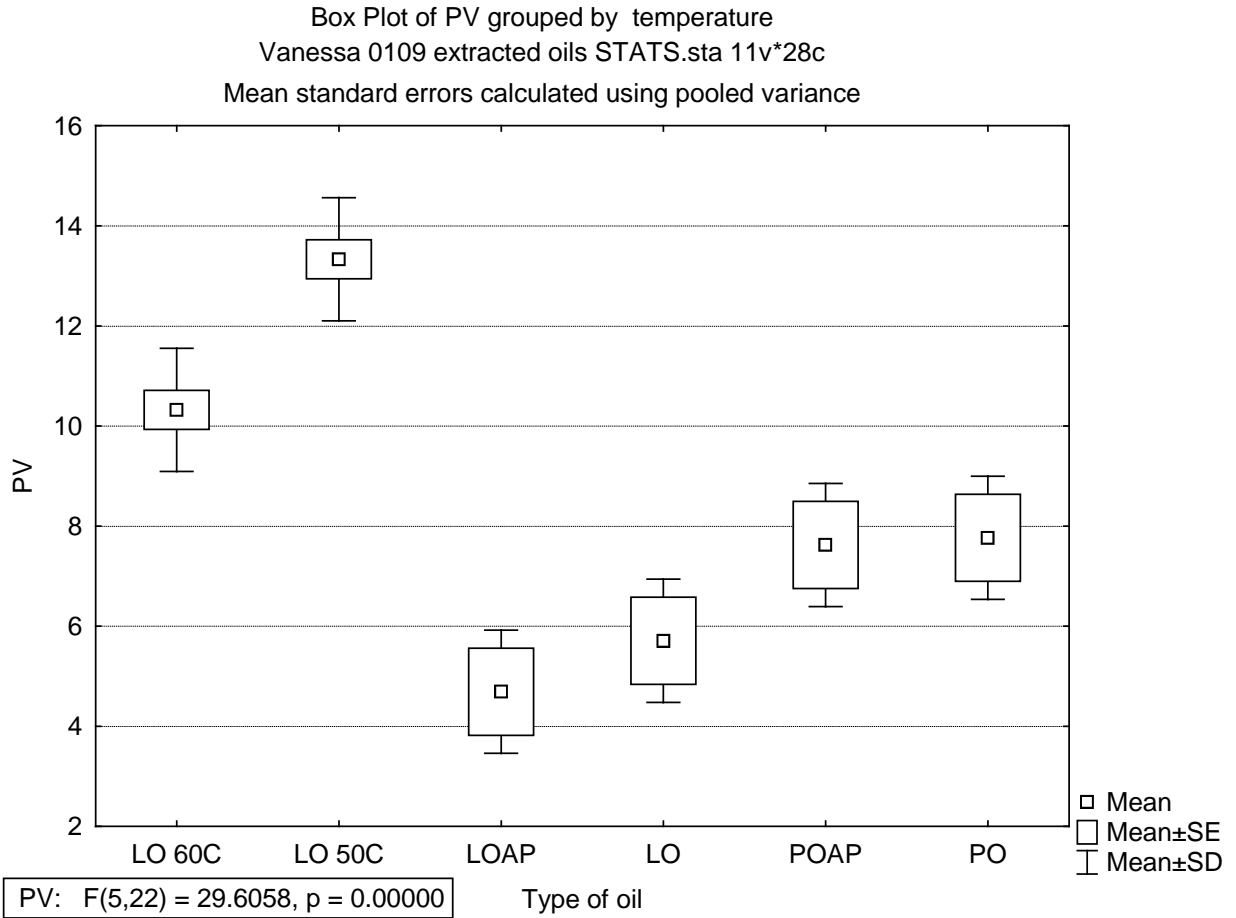
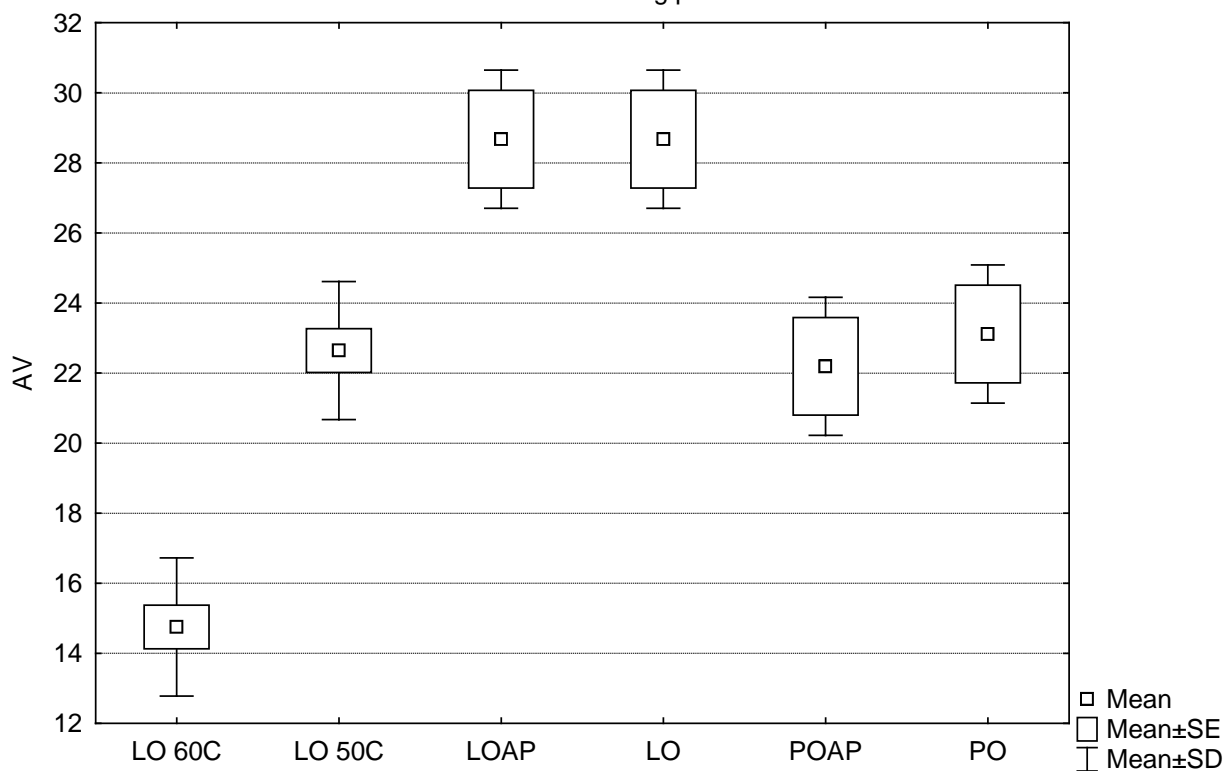


Figure 10. Peroxide values (meq/ Kg) in liver oils rendered in the laboratory and commercial oils. LO 50C and 60C denote liver oils rendered in the laboratory at given temperatures. PO and LO denote pollock oil and pollock liver oil produced commercially by American Seafoods. AP ascorbyl palmitate.

Box Plot of AV grouped by temperature
 Vanessa 0109 extracted oils STATS.sta 11v*28c
 Mean standard errors calculated using pooled variance



AV: $F(5,22) = 34.0646, p = 0.00000$ temperature

Figure 11. Anisidine values in liver oils rendered in the laboratory and commercial oils. LO 50C and 60C denote liver oils rendered in the laboratory at given temperatures. PO and LO denote pollock oil and pollock liver oil produced commercially by American Seafoods. AP ascorbyl palmitate.

Table 13. Average fatty acid profiles of liver oils rendered in the laboratory

	LO 60C (mg/g oil)	LO 50C (mg/g oil)	LO 60C (% w/w)	LO 50C (% w/w)
14:0	42.56	42.30	5.43	5.46
14:1 ω5	0.54	0.41	0.06	0.06
15:0	1.27	1.29	0.16	0.17
Iso or Ante iso16:0	1.58	1.58	0.20	0.20
16:0	68.47	71.02	8.73	9.17
16:1 ω9	0.87	1.20	0.11	0.16
16:1 ω7	83.59	83.60	10.69	10.80
16:1 ω5	2.32	2.28	0.30	0.30
Ante iso17:0	6.88	6.83	0.88	0.88
17:0	5.07	4.97	0.65	0.64
Unknown	5.26	5.42	0.67	0.70
17:1 ω9	0.00	0.08	0.00	0.01
Iso or Ante iso18:0	8.90	8.63	1.14	1.11
18:0	10.00	10.21	1.28	1.32
18:1 ω11	0.97	1.15	0.12	0.15
18:1 ω9 <i>trans</i>	17.23	16.82	2.20	2.17
18:1 ω9 <i>cis</i>	50.06	52.59	6.40	6.79

18:1 ω7	29.85	30.59	3.82	3.95
18:2 ω6 <i>trans</i>	4.08	3.99	0.52	0.51
18:2 ω6 <i>cis</i>	3.41	3.43	0.44	0.44
18:3 ω6	2.48	2.57	0.32	0.33
18:3 ω3	1.55	1.41	0.20	0.18
18:3 ω4	8.59	8.57	1.10	1.11
18:4 ω3	2.44	2.39	0.31	0.31
20:1 ω11	135.66	130.05	17.34	16.80
20:1 ω9	34.23	32.93	4.38	4.25
20:1 ω7	2.25	2.25	0.29	0.29
20:3 ω6	0.43	0.44	0.05	0.05
20:4 ω3	2.25	2.24	0.29	0.29
20:5 ω3	75.68	76.14	9.68	9.84
22:1 ω11	113.18	108.01	14.49	13.95
22:1 ω9	7.36	7.02	0.94	0.91
22:1 ω7	2.25	2.11	0.29	0.27
22:2 ω6	3.98	3.98	0.51	0.51
22:5 ω3	5.71	5.54	0.73	0.72
22:6 ω3	38.07	37.05	4.87	4.79
24:1 ω9	2.70	2.54	0.35	0.33
Σ FA identified	781.63	773.67	99.91	99.93
Σ Unknown FA	5.26	5.42	0.67	0.70
Σ SAT (S)	144.72	146.85	18.46	12.39
Σ MUFA	483.06	473.64	61.78	60.85
Σ PUFA (P)	148.58	147.76	19.00	19.08
P/S	1.03	1.01	1.03	1.54
Σ ω-3	119.98	119.24	15.35	16.12
Σ ω-6	14.29	14.40	1.82	6.64
ω-3/ω-6	8.43	8.30	8.44	2.43

LO liver oil; SAT saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids