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Ethanol extraction of phospholipids from herring roe – optimization and co-extraction of unwanted compounds

Norwegian spring-spawning herring (*Clupea harengus*) represents one of the larges fish stocks in the world with total landings to Norwegian processors in 2020 and 2021 of 416,000 and 490,000 tons, respectively. Fishing season is October-February and coincides with the gonad maturation and spawning season in February-March. Mature roe is collected in February and marketed for use in different roe products. However, there is a huge potential for improved valorization of unmature roe. Herring roe contains high levels of phospholipids and n-3 PUFAs making it an interesting raw material for production of lipid extracts targeting the health food and nutraceutical market.

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Materials and Methods

Immature roe from Norwegian spring spawning (NSS) herring (Clupea harengus) captured beginning of February 2020, was kindly provided by Pelagia AS, Bergen, Norway. The roe was block frozen and stored at -23 °C until thawing at ambient temperature and spray drying at Nofima.

A 2-factorial central composite design (CCD) and response surface methodology was used to optimize the ethanol extraction process variables, temperature (20-60 °C) and water content in the ethanol phase (0-40%). Spray dried roe was contacted with aqueous ethanol under standardized mixing conditions for 5 minutes followed by filtration. Comparison of direct extraction of wet roe and spray dried roe was performed using the optimized conditions.

Optimization of aqueous ethanol extraction

Water content in the solvent phase played a major role influencing total extract and phospholipids yield, and co-extraction of salt and protein (Figure 1). No significant effect of temperature was observed. Best single step phospholipids yield (83%) can be obtained using 11.3% water content in the ethanol phase. Increased swelling of roe and retention of solvent in the filter cake can explain the reduced yield compared to chloroform-methanol extraction. Including this loss at optimal conditions gives 100% yield and reflects the potential for increased yield by washing of the filter cake (Figure 1). Co-extraction of salt and protein followed a close to linear and exponential relationship, respectively, to water content in the ethanol phase.

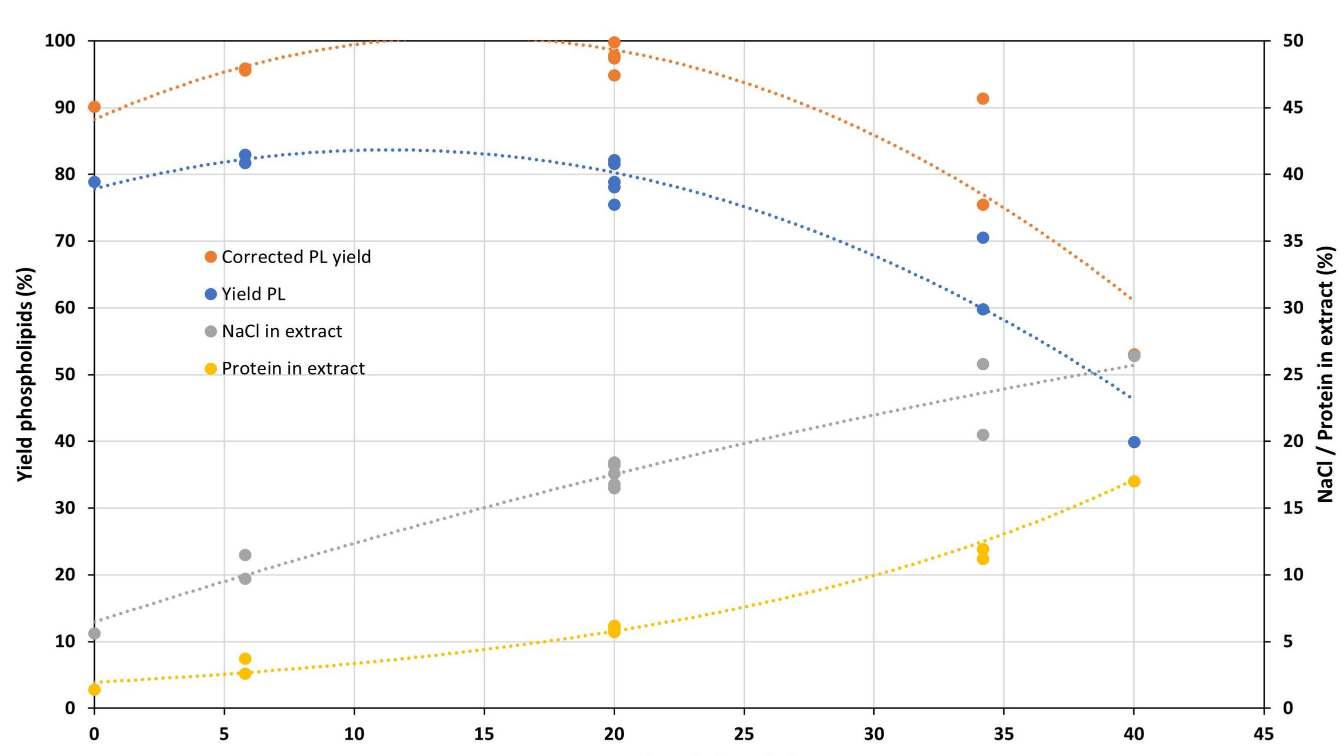

Figure 1 Obtained and corrected (including PLs from entrained solvent in the filter cake) yields of PLs and co-extraction of salt (NaCl) and protein depending on water content in the ethanol phase. Dashed lines are based on models given in Table 1

Response modelling based on level of water in the ethanol phase showed good fitting to the measured responses (Table 1). Estimated optimum level of water in the ethanol phase varied between 5,0 – 19,6% and reflects the polarity of the respective phospholipids.

Table 1 Response surface models for yield of crude extract, total and individual phospholipids (PLs), and estimated optimum water content in the ethanol phase based on the respective models.

PC – phosphatidyl-choline
1-LPC – 1-lysophosphatidyl-choline
2-LPC – 2-lysophosphatidyl-choline
PI – phosphatidyl-inositol
PE – phosphatidyl-ethanolamine
LPE - lyso phosphatidyl-ethanolamine
APE – N-acyl phosphatidyl-ethanolamine
SPH - sphingomyelin

	2. order polynomial				Optimum
Response	X^2	X	Constant	R^2	(water% ethanol phase)
Lipid extract	-0,002	0,067	5,48	0,81	13,9
Total PL	-0,046	1,035	77,8	0,89	11,3
PC	-0,037	0,718	78,2	0,88	9,7
1-LPC	-0,053	1,576	100,7	0,74	14,8
2-LPC	-0,023	0,556	103,6	0,63	11,9
PI	-0,437	17,02	90,8	0,78	19,5
PE	-0,062	1,590	66,4	0,86	12,7
LPE	-0,129	5,077	82,0	0,69	19,6
APE	-0,168	4,877	65,2	0,81	14,5
SPH	-0,056	0,560	109,2	0,88	5,0

Comparison of wet vs. spray dried roe extraction

Comparison of dewatering of the roe (spray drying) before or as part of the extraction process, revealed 25% higher yield and significant advantages of the former procedure (Figure 2). The developed protocol gives a lipid extract containing 65-70% phospholipids and 31% n-3 PUFAs. Persistent organic pollutants (POPs) and arsenic were extracted quantitatively, however, heavy metals (Cd, Pb, Hg) only partly (Table 2). Levels of undesirable compounds were well below current legislation levels.

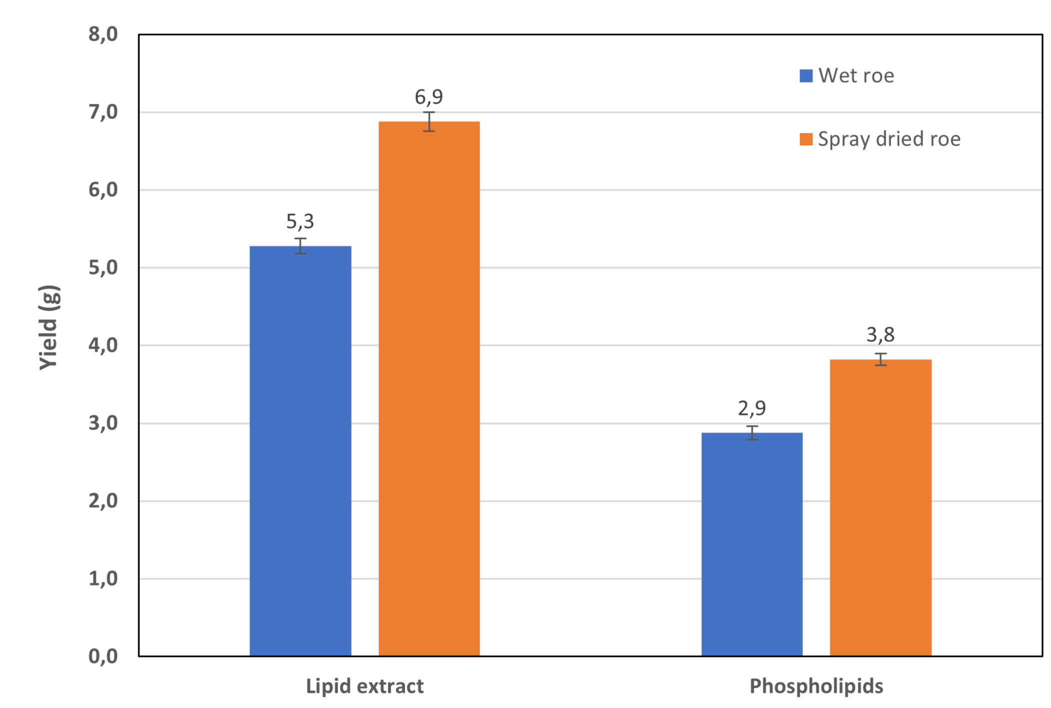

Figure 2 Yield of lipid extract and phospholipids based on extraction of 30 g DM of wet and spay dried herring roe.

Table 2 Co-extraction of POPs and heavy metals on dry matter and lipid basis using 11.3% aqueous ethanol.

	DM basis		Lipid basis		
POPs (upper bound level)	**Freeze dried roe**	**Lipid extract**	**Freeze dried roe**	**Lipid extract**	**Max level [1)]**
PCDD/F-TEQ2005 (ng/kg)	0,195	0,869	1,086	0,998	1,75
DL-PCB-TEQ2005 (ng/kg)	0,155	0,710	0,864	0,816	---
PCDD/F+DL-PCB-TEQ2005 (ng/kg)	0,350	1,579	1,950	1,815	6,0
NDL-PCBs (µg/kg)	2,3	10,3	12,9	11,8	200
Heavy metals (mg/kg)					
Arsenic (As)	1,4	6,0	7,8	6,9	---
Cadmium (Cd)	0,022	<LOQ	0,123	<LOQ	1,0
Lead (Pb)	0,0028	<LOQ	0,016	<LOQ	3,0
Mercury (Hg)	0,017	<LOQ	0,095	<LOQ	0,1
Selenium (Se)	2,9	3,0	16,2	3,4	---

1) Commission regulation (EC) No. 1881/2006.
PCDD – Polychlorinated dibenzodioxin; PCDF - Polychlorinated dibenzofuran; DL-PCB - Dioxin-like polychlorinated biphenyl; NDL-PCB - Non-dioxin-like polychlorinated biphenyl (sum PCB6: PCB-28, 52, 101, 138, 153 and 180); TEQ – toxic equivalents

Conclusions

Effective extraction of spray dried herring roe can be performed based on aqueous ethanol an gives a lipid extract with high levels of phospholipids (65-70%) and n-3 PUFAs (31%). Optimum water content for lipid extract and phospholipids yield was 13,9% and 11,3%, respectively. Practical yield of phospholipids at optimum conditions was 83% with loss explained by retention of solvent in the filter cake. Co-extraction of salt and protein depends on water content in the ethanol phase.

Extraction of wet roe gave 25% lower yields and require excess use of ethanol to obtain optimal conditions. POPs and heavy metals were co-extracted quantitatively, however, gives levels well below regulatory levels.

References:

1) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L364, 5-24.

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