Supporting Information for

Two-dimensional $^{31}P,^{1}H$ NMR Spectroscopic Profiling of Phospholipids in Cheese and Fish

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1. Detection of phospholipids in the neutral liquid-liquid-extraction faction n-nn

Liquid-liquid-extraction (LLE) of the cheese lipids resulted in the discrimination of diphosphatidylglycerol cardiolipin (CL). This nonpolar PL and a further unknown phosphorus compound (u_n) were detected in the nonpolar LLE fraction of cheese fat (organic brie). Cardiolipin produced $^{1}H,^{31}P$ cross couplings of the backbone and head group (connection center of the dimers) protons (Figure S1). The unknown phosphorus compound showed one single cross coupling similar to phosphatidic acid (Table 2). The polarity of phosphatidic acid and pH experiments rule out this possibility. This compounds u_n could not identified yet.

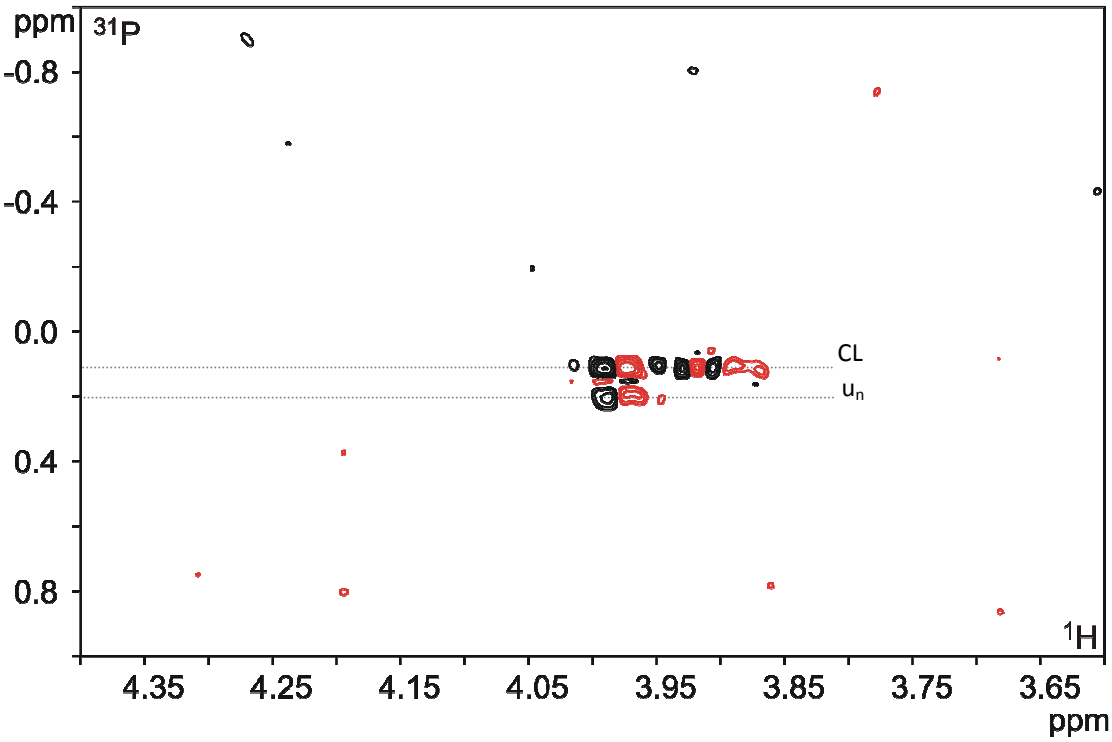

Figure S1. 2D $^{1}H,^{31}P$ NMR spectrum of the nonpolar cheese fat fraction after LLE (n-nn). Cardiolipin (CL) and an unknown phosphorus compound (u_n) were detected.

2. Investigating the unknown phosphorus compound “u” in fish

Traces of phosphatidylethanolamine (PE) and alkyl ether-linked phosphatidylethanolamine (PEe) traces in the 2D COSY NMR spectrum did not match the unknown compound (u) in fish (Figure S2). Phosphatidylethanolamine plasmalogen (PEp) could also be excluded because of ^{1}H chemical shifts at 4.04 and 3.96 ppm [1]. The ^{1}H chemical shifts of lysophosphatidylethanolamine (LPE) were also shifted and thus could be excluded because of the different ^{31}P chemical shift of 0.43 ppm (not shown).

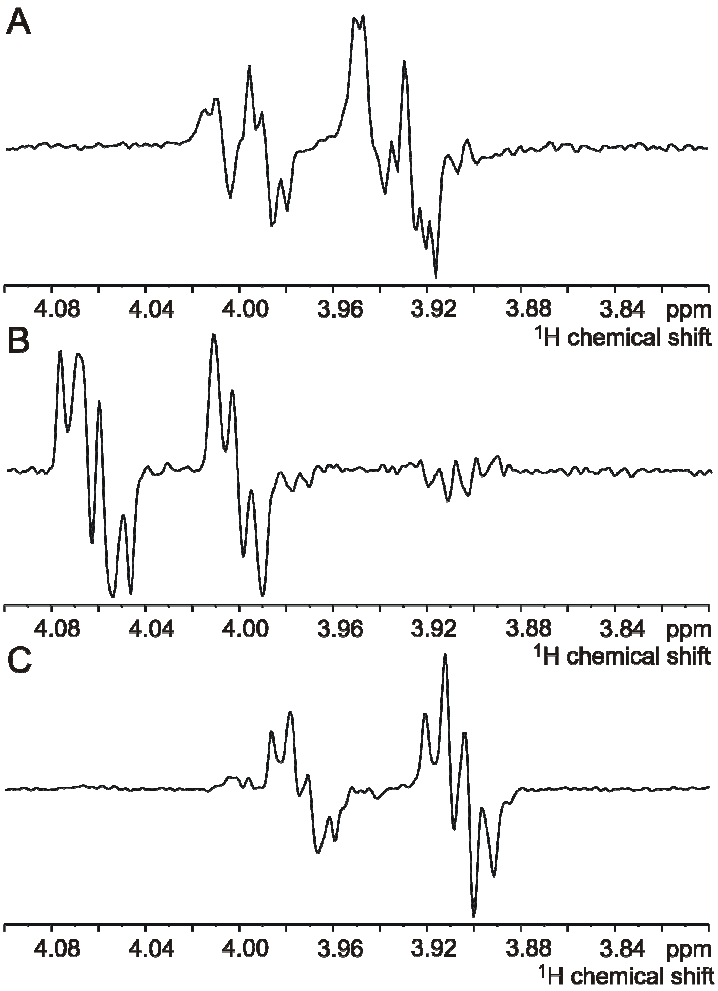

Figure S2. ^{1}H traces from a 2D COSY NMR spectrum with (A) Unknown compound in fish (^{31}P shift -0.02 ppm, Fig 2b), (B) phosphatidylethanolamine (PE) trace for comparison and (C) alkyl ether-linked phosphatidylethanolamine (PEe) trace for comparison.

3. Calculation mode of the quantification of phospholipids in cheese and fish

The total PL amount of each sample was determined by means of the intergral of all peaks in the 1D NMR spectrum. An integral of 1 corresponded to 0.8 µmol (based on the internal standard TPP). The resulting µmol values were converted into mg amount based on average molecular weights of 750 g/mol for cheese (M_{cheese}) and 790 g/mol for fish (M_{fish}), Table S1. Because the PL were distributed over six different fractions by liquid-liquid (Figure 1), two cheese and one fish sample were chosen for which all six fractions were quantified by NMR. For all other samples, only the polar, two-fold re-extracted extract (p-pp) was quantified (Table S1). The total PL amounts by 1D NMR were compared to the SPE GC/MS results, described by Hauff and Vetter of the same samples [2]. The PL amount is in the same range except cheese where higher PL contents were determined with GC/MS [2]. The reason could be the polar neutral lipids with low molecular weight in milk fat, which could also be determined by SPE GC/MS [2].

Table S1. Quantification data of total PL amounts in fish oil and cheese fat samples

		tube			LLE fraction	fat extract	NMR		GC/MS [2]
samples	LLE fraction	m tube [mg] (total LLE)	total PL [μmol]	PL [mg]*	PL [mg] total	total [mg]	LLE PL [%]	total PL [%]	total PL [%]
				cheese					
butter-cheese	p-pp	29.4 (33.4)	1.58	1.19	1.35	444		0.31	0.57
organic brie	Sum fractions					1034		0.35	0.68
	p-pp	26.6 (30.9)	1.65	1.23	1.43		0.14		
	n-nn	30.0 (816.1)	0.029	0.022	0.592		0.060		
	n-p	27.5 (59.4)	0.53	0.39	0.853		0.082		
	n-pp	29.1 (91.4)	0.15	0.11	0.357		0.035		
	p-n	29.6 (47.1)	0.20	0.15	0.240		0.023		
	p-nn	7.6 (7.6)	0.17	0.13	0.130		0.013		
	ASE extract	31.4 (1050.0)	0.15	0.11	3.76	1050		0.36	0.68
bovine mozzarella	Sum fractions					1043		0.30	0.53
	p-pp	12.1 (15.8)	2.55	1.91	2.49		0.24		
	n-nn	30.4 (849.1)	-						
	n-p	32.7 (37.4)	0.49	0.37	0.418		0.040		
	n-pp	32.6 (70.1)	0.06	0.05	0.103		0.010		
	p-n	36.2 (36.2)	0.14	0.11	0.106		0.010		
	p-nn	3.1 (3.1)	0.08	0.06	0.062		0.006		
	ASE extract	30 (735)	0.03	0.02	0.473	735		0.06	0.53
edam cheese	p-pp	16.6 (22.3)	1.45	1.09	1.463	818		0.18	k.a.
				fish					
salmon rainbow trout	p-pp	30.0 (102.0)	4.42	3.49	11.87	268		4.60	6.03
	Sum fractions					770		12.20	10.30
	p-pp	30.1 (289.1)	8.11	6.41	61.55		8.00		
	n-nn	33.2 (266.8)	1.67	1.32	10.59		1.38		
	n-p	31.9 (83.3)	6.68	5.28	13.79		1.79		
	n-pp	31.3 (74.3)	3.21	2.54	6.04		0.78		
	p-n	31.0 (31.0)	2.20	1.74	1.74		0.23		
	p-nn	9.5 (9.5)	0.67	0.53	0.53		0.07		
	ASE extract	30.1 (308.5)	4.95	3.91	40.04	309		12.98	10.30
gilthead seabream	p-pp	29.5 (312.8)	2.10	1.66	17.61	1083		1.66*	10.60

* M_{cheese} 750 g/mol; M_{fish} 790 g/mol

4. Distribution of phospholipids in cheese and fish after liquid-liquid extraction

The distribution of PLs in each fraction after liquid-liquid extraction [% of total PL] are shown in Table S2. By means of these results, discrimination effects are observable, if a liquid-liquid enrichment of cheese fat and fish oil samples were performed. As mentioned before, the minor PLs in cheese fat can not be determined without any enrichment procedure.

Table S2. Weight of all liquid-liquid-extraction fractions of cheese fat and fish oil samples and their distribution of PL (% of total PL) in each fraction.

sample	fraction	weight of each fraction [mg]	PLs [mg] / fraction	PLs [mg/g fat]	PLs [% of total PL] in fraction*
cheese					
butter cheese	p-pp	33.4	1.35	3.45	PC (43.0%), PI (4.1%), LPC (2.5%), SM (21.6%), PE (11.7%), compound u (5.4%), DHSM (4.1%), PA (7.4%)
	n-nn	652.3	-	-	-
organic brie	p-pp	30.9	1.43	1.39	PC (66.0%), PI (4.7%), LPC (3.1%), SM (12.9%), PE (2.3%), compound u (4.5%), DHSM (2.0%), PA (1.1%), PG (0.2%)
	n-nn	816.1	0.592	0.35	CL (64.4%), compound u_n (35.6%)
	n-p	91.4	0.853	0.82	PC (59.4%), PI (3.4%), LPC(2.4%), SM (14.2%), compound u (13.6%), PA (0.7%)
	n-np	59.4	0.240	0.57	PC (46.9%), SM (14.1%), PE (7.7%), compound u (16.0%), PA (15.1%)
	p-n	47.1	0.357	0.23	PC (60.0%), SM (13.6%), PE (6.9%), compound u (9.2%), PA (10.4%)
	p-pn	7.6	0.130	0.13	PC (69.5%), PI (3.0%), LPC (4.0%), SM (17.7%), compound u (5.8%)
bovine mozzarella	p-pp	15.8	2.49	2.39	PC (42.3%), PI (7.6%), LPC(3.3%), SM (20.6%), compound u (4.2%), DHSM (4.6%), PA (13.2%), LPA(1.1%)
	n-nn	849.1	0.072	0.07	compound u_n
	n-p	37.4	0.418	0.10	PC (46.8%), PI (2.4%), LPC (6.0%), SM (28.6%), compound u (1.7%), DHSM (6.3%), PA (6.5%), LPA (1.5%)
	n-np	36.2	0.106	0.40	PC (67.2%), SM (32.8%)
	p-n	70.1	0.103	0.10	PC (46.1%), LPC (4.1%), SM (32.2%), DHSM (11.4%), PA (6.1%)
	p-pn	3.1	0.062	0.06	PC (48.9%), SM (38.0%), DHSM (5.2%), PA (8.0%)
edam cheese	p-pp	22.3	1.463	1.79	PC (44.9%), PI (5.3%), LPC (3.6%), SM (23.7%), PE (3.9%), compound u (5.2%), DHSM (6.1%), PA (5.5%)
	n-nn	683.8			-
sample	fraction	weight of each fraction [mg]	PLs [mg] / fraction	PLs [mg/g fat]	PLs [% of total PL] in fraction*

Table S2, continued

sample	fraction	weight of each fraction [mg]	PLs [mg] / fraction	PLs [mg/g fat]	PLs [% of total PL] in fraction*
fish					
salmon	p-pp	102.0	11.87	44.23	PC (25.7%), PCe (10.3%), PI (10.5%), LPC (25.5%), LPCp (11.6%), SM (2.5%), PA (2.4%), PG (1.0%), LPA (3.6%)
	n-nn	66.1	-	-	-
rainbow trout	p-pp	289.1	61.55	79.97	compound a (10.6%), PC (31.7%), PCe (4.0%), PI (6.5%), LPC (35.6%), LPCp (4.1%), SM (2.8%), PA (0.9%), LPA (1.5%)
	n-nn	266.8	10.59	13.76	-
	n-p	83.3	13.79	7.84	compound a (4.0%), PC (48.3%), PCe (4.8%), PI (4.8%), LPC (22.1%), LPCp (2.8%), SM (6.5%), PA (1.4%), LPA (1.8%)
	n-np	31.0	1.74	17.92	PC (69.7%), PCe (6.9%), PI (2.8%), LPC (7.2%), LPCp (0.8%), SM (7.4%), PA (2.3%), LPA (0.8%)
	p-n	74.3	6.04	2.26	PC (62.9%), PCe (7.3%), PI (3.1%), LPC (14.3%), LPCp (2.2%), SM (6.9%), SM (0.7%), PA (1.5%), LPA (0.9%)
	p-pn	9.5	0.53	0.69	compound a (3.9%), PC (56.9%), PCe (5.5%), PI (1.7%), LPC (18.3%), LPCp (2.6%), SM (7.8%), PA (0.8%), LPA (1.4%)
gilthead seabream	p-pp	312.8	17.61	16.26	PC (43.6%), PCe (3.8%), PI (7.5%), LPC (37.6%), LPCp (2.0%), SM (1.7%), PA (0.7%), LPA (1.3%)
	n-nn	154.7	-	-	-

* with PC, phosphatidylcholine; PCe, alkyl ether-linked phosphatidylcholine; LPC, lysophosphatidylcholine; LPCp, lysophosphatidylcholine plasmalogen; PE, phosphatidylethanolamine; PEe, alkyl ether-linked phosphatidylethanolamine; PI, phasphatidylinositol; SM, sphingomyelin; DHSM, dihydrosphingomyelin; PA, phosphatidic acid; PG, phosphatidylglycerol; LPA, lysophosphatidic acid; CL, cardiolipin; u, unknown in polar fraction p-pp; compound u_n, unknown in nonpolar fraction n-nn; compound “a” was ambiguous, PC with high degree of unsaturated fatty acids bonded or PCp.

References

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[2] Hauff, S.; Vetter, W. Quantification of branched chain fatty acids in polar and neutral lipids of cheese and fish samples. *J. Agric. Food Chem.* **2010,** *58,* 707-712.