

SUPPLEMENTARY MATERIAL

QuEChERS-based methodology for the screening of Alkylphenols and Bisphenol A in dairy products

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Table S1. Characteristics of the selected dairy products.

A/A	Food Commodity	Fat (%)	Type of Packaging
1	Fresh cow milk1	3.5	paper
2	Fresh cow milk2	1.5	paper
3	Pasteurized milk1	3.5	paper
4	Pasteurized milk2	0	paper
5	Chocolate milk	1	paper
6	Condensed milk	7	aluminum
7	Coconut milk	7.6	aluminum
8	Cream1	35	paper
9	Cream2	17	paper
10	Organic yoghurt	3.9	plastic
11	Goat yoghurt	4	plastic
12	Cow yoghurt1	3.9	plastic
13	Cow yoghurt2	2	plastic
14	Cow yoghurt3	0	plastic
15	Strained yoghurt1	8	plastic
16	Strained yoghurt2	0	plastic
17	Yoghurt dessert	1	plastic
18	Sheep yoghurt	6	plastic
19	Goat-sheep yoghurt	4	plastic
20	Infant yoghurt	4.4-4.6	plastic
21	Powdered milk	0.8	paper
22	Hydrolyzed protein infant formula	3.5	aluminum
23	Organic infant formula	2.7	paper
24	Lactose free infant formula	3.6	aluminum
25	Condensed infant milk	3.1	aluminum
26	Ice cream1	3.5	plastic
27	Ice cream2	0	plastic

Table S2. Data from LC-Orbitrap/MS² analysis of the target EDCs.

Chemical Compounds	Orbitrap MS				Orbitrap MS ²
	Precursor Ion	Theoretical Mass	Experimental Mass	Mass Error	Fragment Ion
		(<i>m/z</i>)	(<i>m/z</i>)	(ppm)	(NCE 45%)*
BPA	C ₁₅ H ₁₅ O ₂ ⁺	227.1078	227.1084	2.64	212.0845
OP	C ₁₄ H ₂₁ O ⁺	205.1598	205.1595	-1.46	133.0062
NP(t)	C ₁₅ H ₂₃ O ⁺	219.1754	219.1750	-1.83	106.0431
4NP	C ₁₅ H ₂₃ O ⁺	219.1754	219.1750	-1.83	106.0431

Table S3. LC-Orbitrap MS parameters under investigation using a fractional factorial design $3^{(4-1)}$ and their coded values.

Factors	Coded Levels		
	(-1)	(0)	(+1)
Injection volume (μL)	5	10	20
Spray voltage (V)	2.5	3.0	3.5
NH ₃ (%)	0	0.05	0.1
Oven temperature ($^{\circ}\text{C}$)	20	30	40

Table S4. Plackett–Burman design with the selected factors and their coded values.

Factors	Coded Levels		
	(-1)	(0)	(+1)
(x ₁) Sample amount (mL)	5	7.5	10
(x ₂) Solvent volume (ACN) (mL)	5	10	15
(x ₃) MgSO ₄ (g)	4	5	6
(x ₄) NaCl (g)	0.5	1	2
(x ₅) PSA (mg)	25	37.5	50
(x ₆) C18 (mg)	25	37.5	50
(x ₇) CH ₃ COOH (%)	0	1	2

Experiments	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇
7	-1	+1	+1	-1	-1	+1	-1
3	-1	+1	-1	-1	+1	-1	+1
11	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0
1	-1	-1	-1	+1	+1	+1	-1
8	+1	+1	1	+1	+1	+1	+1
4	+1	+1	-1	+1	-1	-1	-1
10	0	0	0	0	0	0	0
5	-1	-1	+1	+1	-1	-1	+1
6	+1	-1	+1	-1	+1	-1	-1
2	+1	-1	-1	-1	-1	+1	+1

Table S5. ANOVA table generated from the Plackett–Burman design.

Factors	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Squares (MS)	F-Test	p-Value
x ₁	1868.13	1	1868.133	800.628	0.00124
x ₂	7828.13	1	7828.133	3354.914	0.00029
x ₃	8.51	1	8.508	3.646	0.19639
x ₄	565.32	1	565.320	242.280	0.00410
x ₅	56.45	1	56.445	24.191	0.03893
x ₆	59.13	1	59.133	25.343	0.03726
x ₇	1976.63	1	1976.633	847.128	0.00117
Lack of fit	18.17	1	18.165	7.785	0.10803
Pure error	4.67	2	2.333		
Total SS	12385.14	10			

Table S6. The Central Composite Design (CCD) including the selected factors after screening design and the mean recovery (%) used as optimal response.

Factors	Coded Levels			Axial Points ($\alpha=1.682$)	
	(-1)	(0)	(+1)	$-\alpha$	$+\alpha$
(x_1) NaCl (g)	1	1.5	2	0.66	2.34
(x_2) C18 (mg)	25	37.5	50	16.48	58.52
(x_3) CH ₃ COOH (%)	1	1.5	2	0.66	2.34
Runs	X_1	X_2	X_3	Mean Recovery (R%)	
1	1	-1	-1	89.5	
2	1	1	-1	94.8	
3	1	-1	1	87.8	
4	-1	-1	1	81.8	
5	$+\alpha$	0	0	68.5	
6	0	$+\alpha$	0	100.3	
7	0	0	0	102	
8	0	0	$+\alpha$	78.5	
9	-1	1	-1	111.0	
10	0	0	0	108.3	
11	0	0	0	106.0	
12	0	$-\alpha$	0	69.0	
13	$-\alpha$	0	0	95.3	
14	-1	-1	-1	87.5	
15	1	1	1	77.8	
16	0	0	$-\alpha$	89.0	
17	-1	1	1	103.3	

Table S7. ANOVA table generated from the Central Composite Design (CCD).

<i>Factors</i>	<i>Sum of Squares (SS)</i>	<i>Degrees of freedom (df)</i>	<i>Mean Squares (MS)</i>	<i>F-Test</i>	<i>p-Value</i>
(x ₁)NaCl(L)	453.960	1	453.9600	45.30163	0.021369
NaCl(Q)	433.034	1	433.0337	43.21334	0.022368
(x ₂)C18(L)	630.669	1	630.6693	62.93581	0.015520
C18(Q)	307.826	1	307.8260	30.71860	0.031046
(x ₃)CH ₃ COOH (L)	182.391	1	182.3913	18.20121	0.050792
CH ₃ COOH(Q)	345.353	1	345.3529	34.46349	0.027811
x ₁ x ₂ (L)	309.383	1	309.3828	30.87396	0.030897
x ₁ x ₃ (L)	3.445	1	3.4453	0.34381	0.616998
x ₂ x ₃ (L)	37.195	1	37.1953	3.71180	0.193869
Lack of fit	423.187	5	84.6374	8.44615	0.109239
Pure error	20.042	2	10.0208		

Table S8. Mean recoveries obtained from the optimized QuEChERS extraction of the target EDCs from yogurt samples at 10 and 50 ng g⁻¹ fortification levels.

EDCs	Rec ± RSD (%) 10 ng g ⁻¹	Rec ± RSD (%) 50 ng g ⁻¹
BPA	91±6.3	73.8±2.6
OP	102.1±9.1	98.5±8.2
NP	85.1±10.2	105.4±7.3
4NP	93.1±7.6	87.7±8.5

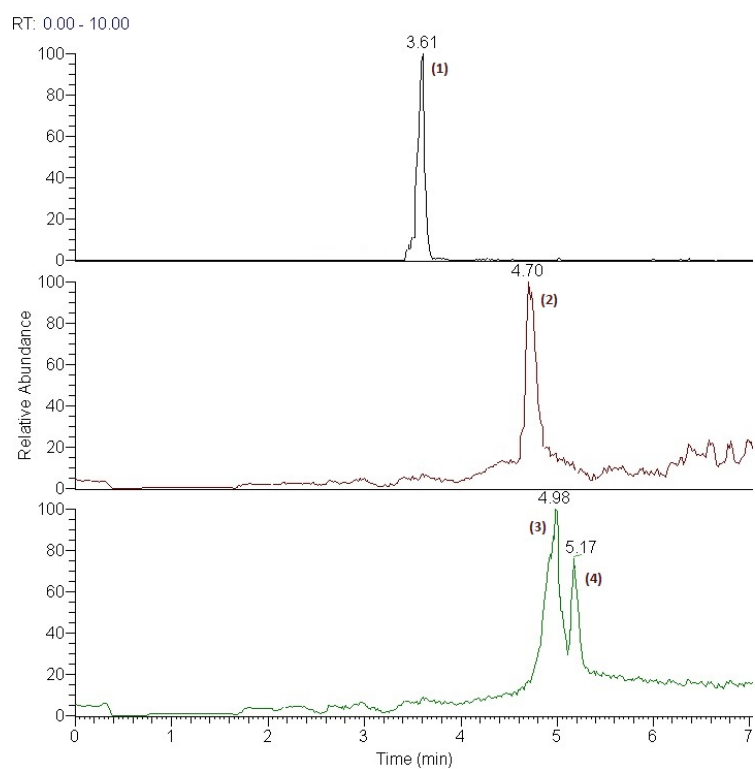


Figure S1. LC-LTQ/Orbitrap MS extracted ion (EI) chromatogram of a standard solution of the selected compounds at a concentration level of $100\ \mu\text{g L}^{-1}$. Peaks are assigned as follows: (1) BPA, (2) OP, (3) NP (t), (4) 4NP.

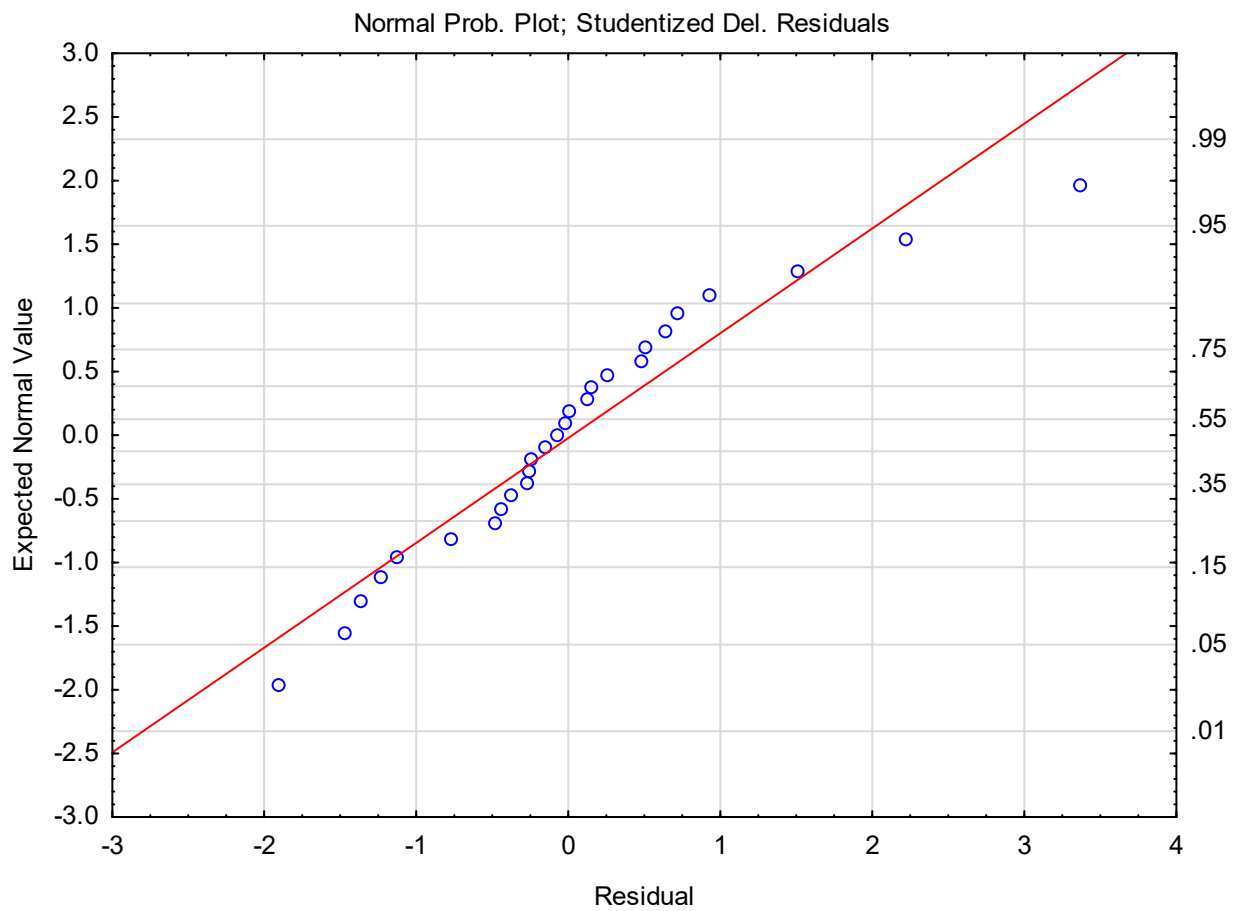


Figure S2. Normal probability plot of the studentized residuals (normality test curve for the fractional factorial design $3^{(4-1)}$).

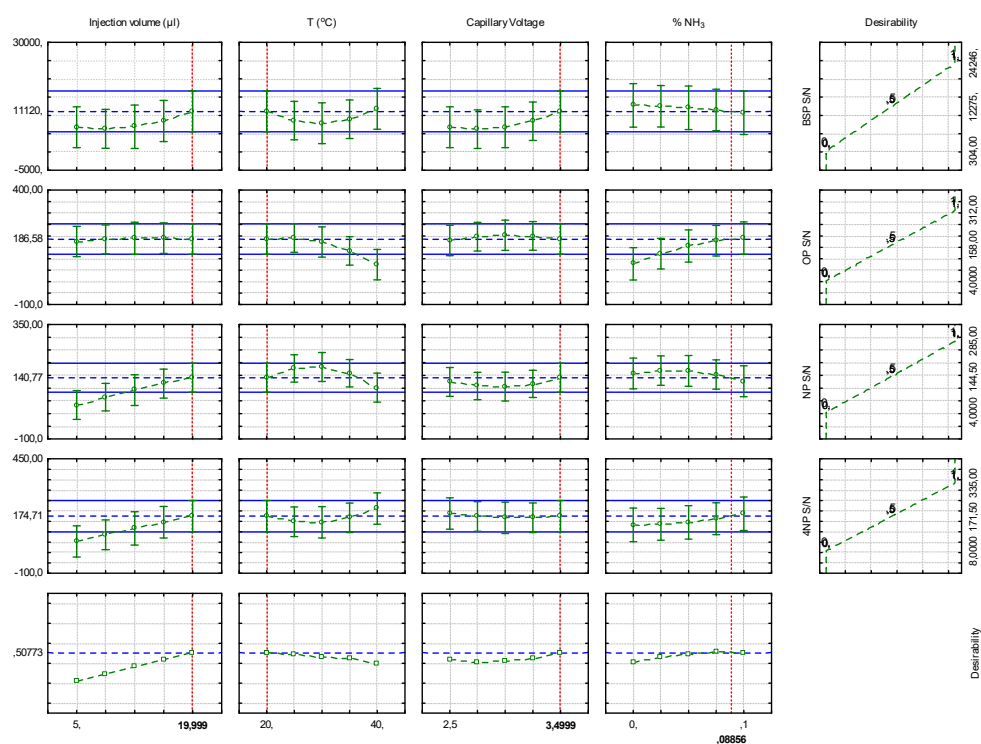


Figure S3. Profile of the predicted values and desirability function of analytes response (S/N). Optimum values are designated by dotted lines.

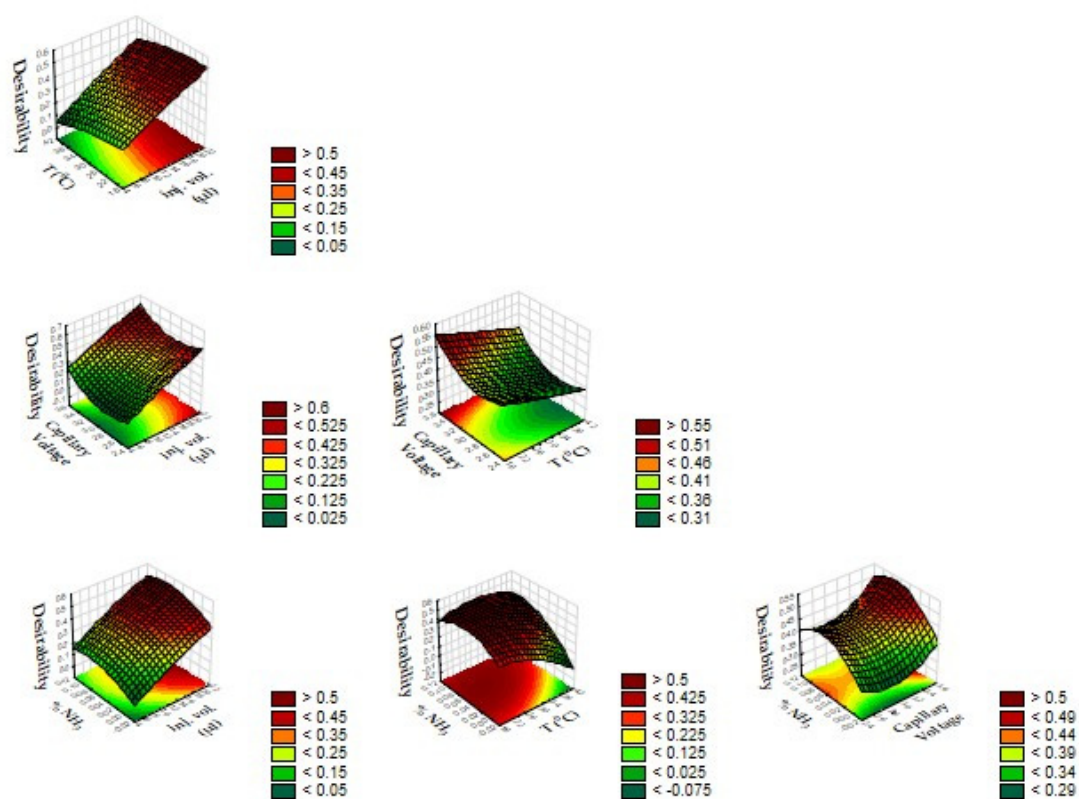


Figure S4. Response surface plots for the fractional factorial design $3^{(4-1)}$.

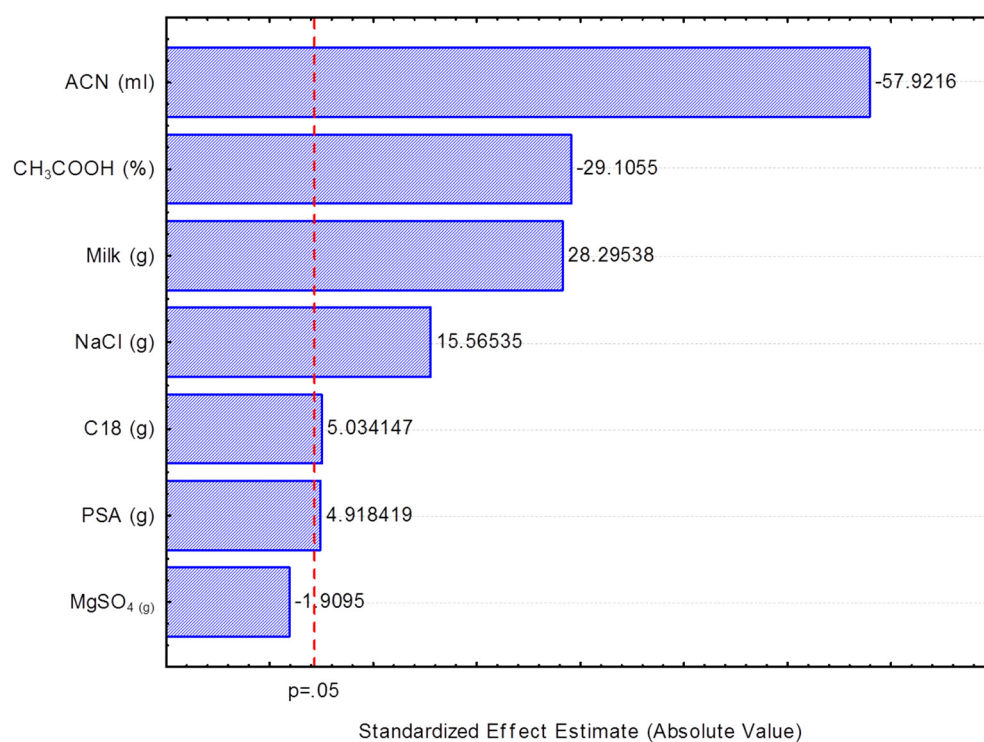


Figure S5. Standardized main effect Pareto chart for the Plackett–Burman design of the screening experiment. Vertical line in the chart defines 95% confidence level.

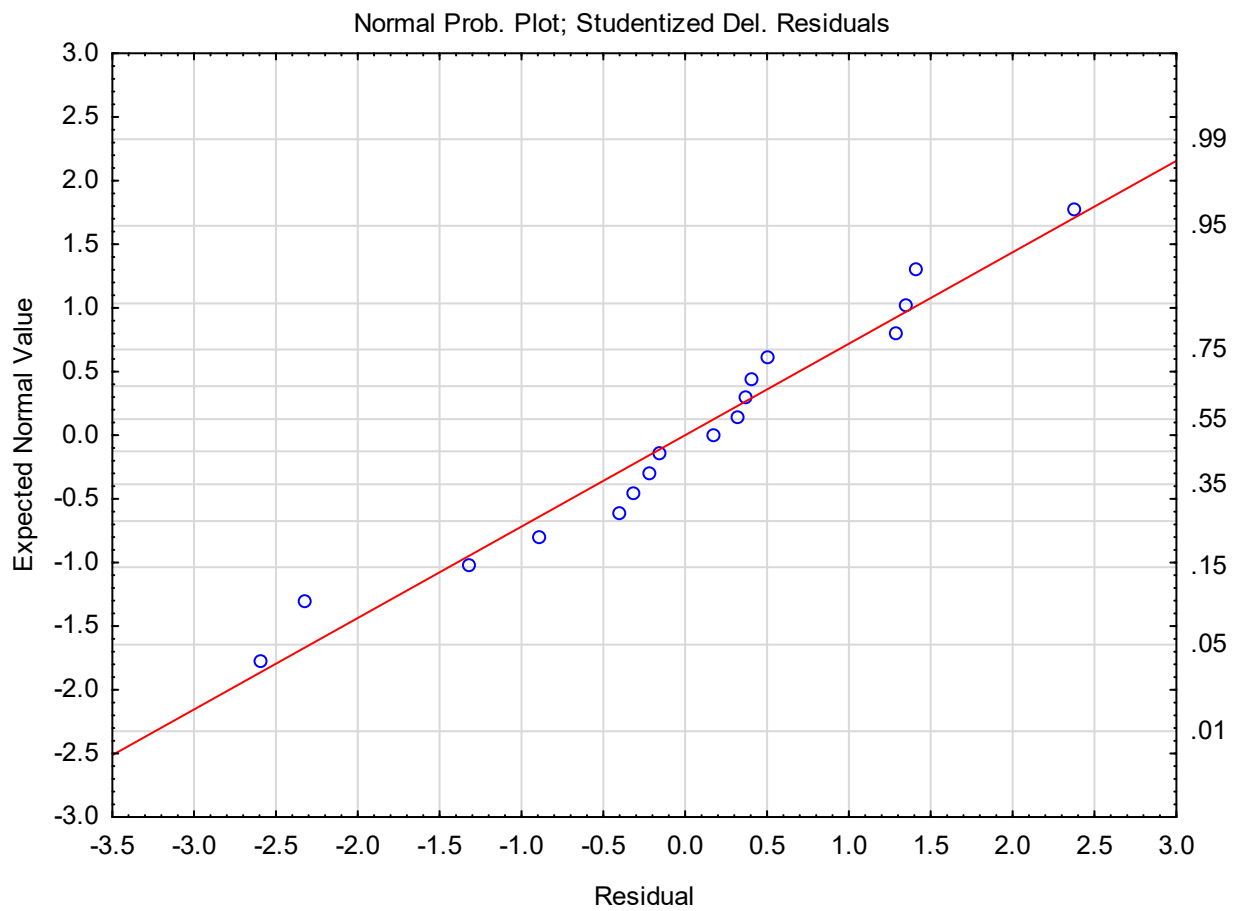


Figure S6. Normal probability plot of the studentized residuals (normality test curve for the central composite design 2^3).

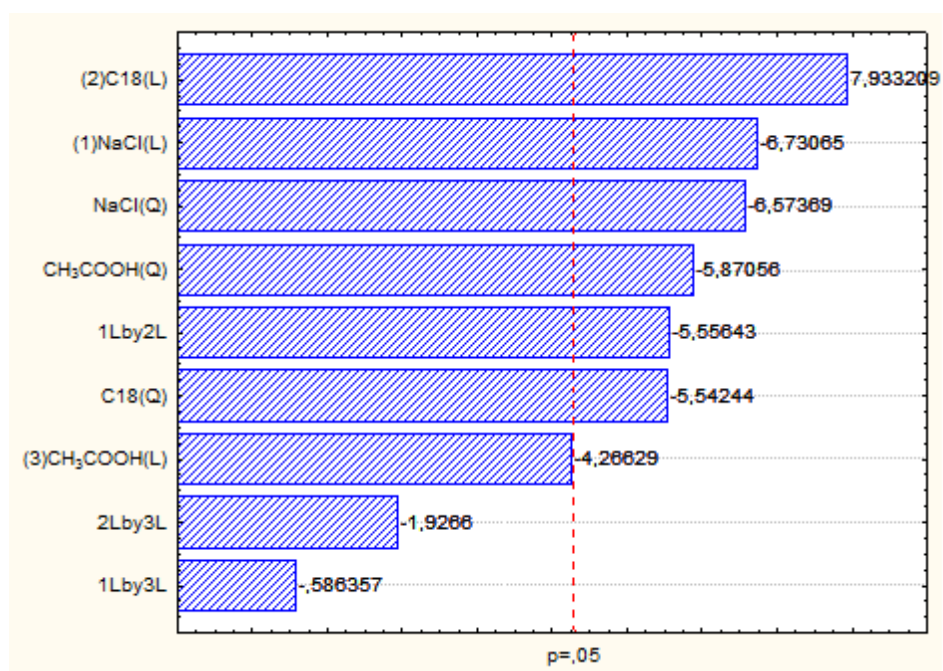


Figure S7. Pareto chart for the Central Composite Design (CCD). Vertical line in the chart defines 95% confidence level.

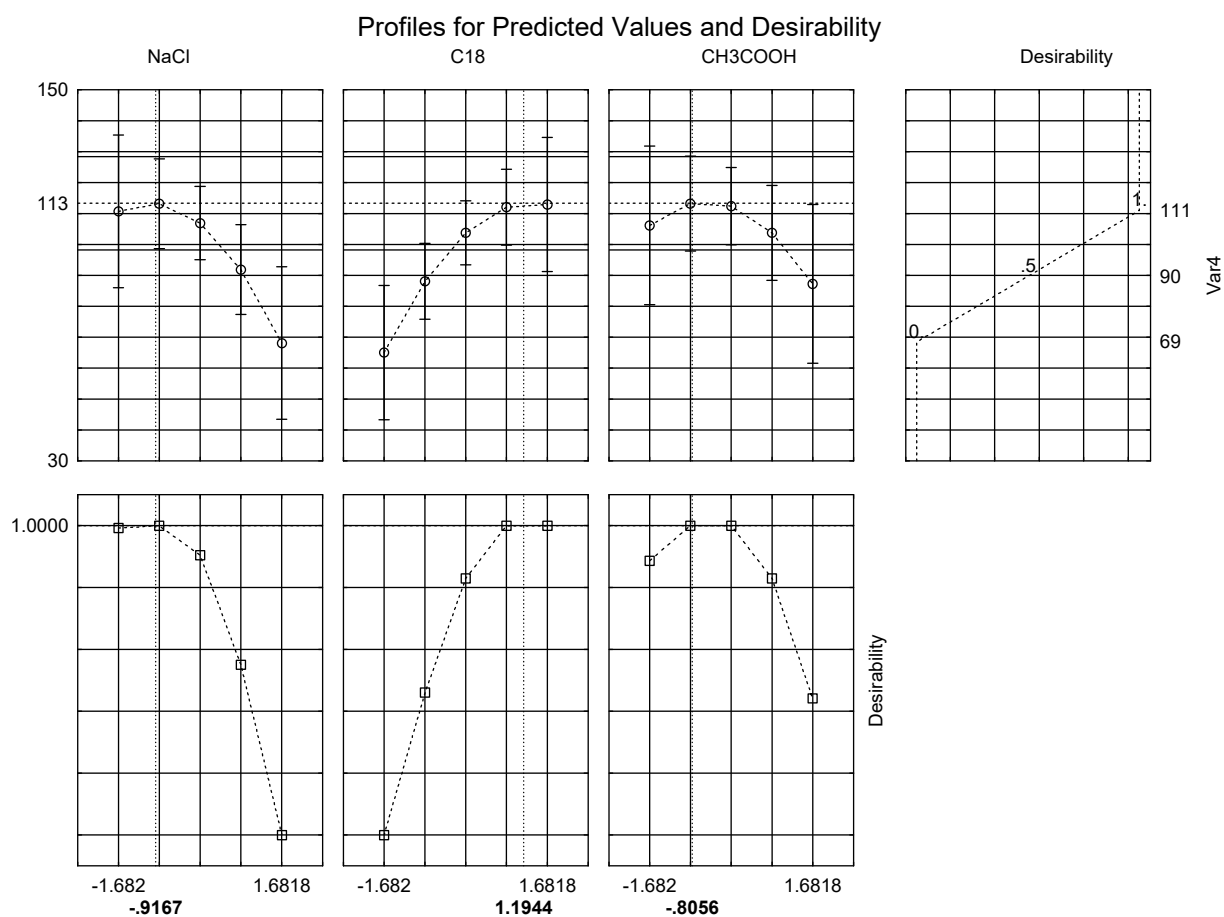


Figure S8. Profile of the predicted values and desirability function of the analytes response (mean recovery %) after CCD employment. The optimal values are designated by dotted lines.

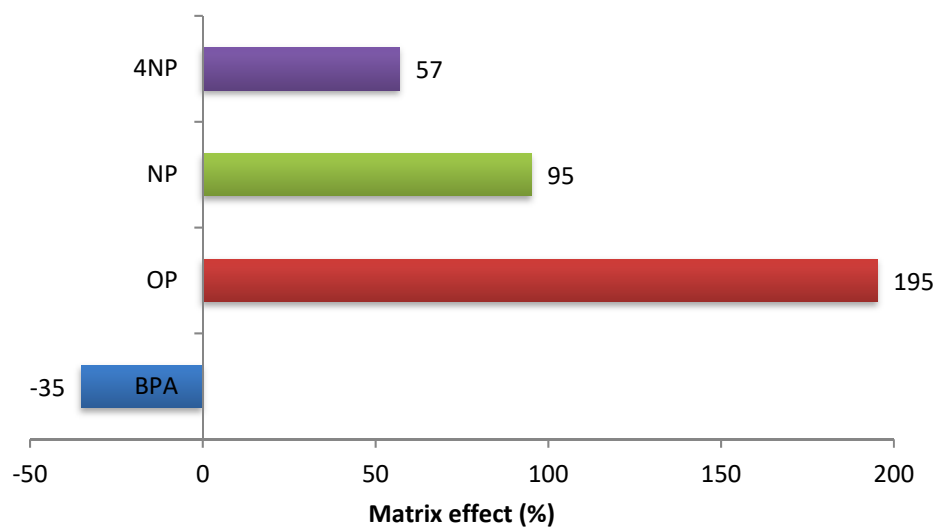


Figure S9. Matrix effect of the EDCs under study, calculated from the slopes of the calibration curves.

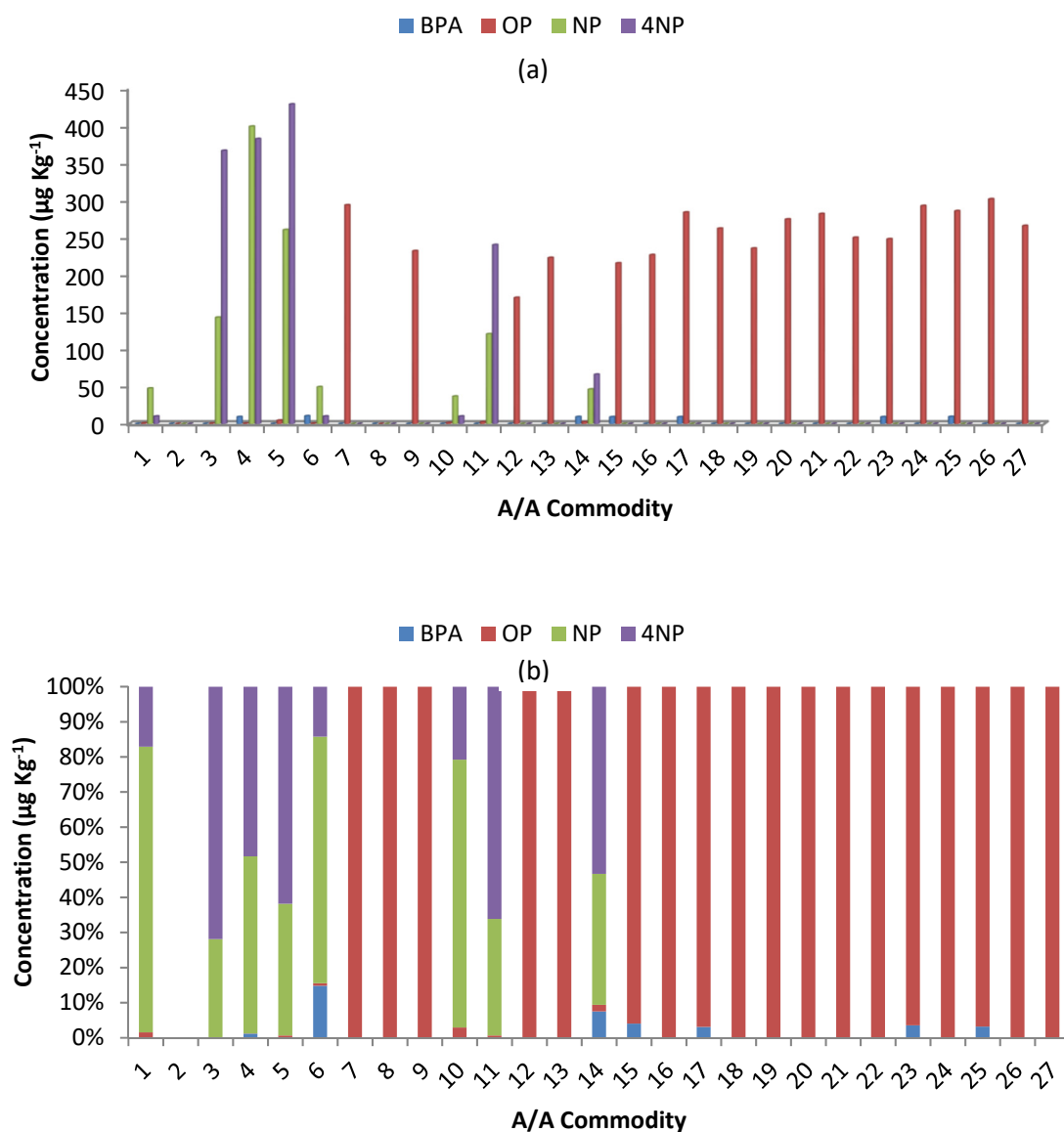


Figure S10. (a) Concentration levels of EDCs residues; (b) percentage concentration patterns in all dairy commodities analyzed by QuEChERS coupled to LC-Orbitrap MS. Numbers of the commodities are assigned according to Table S1.

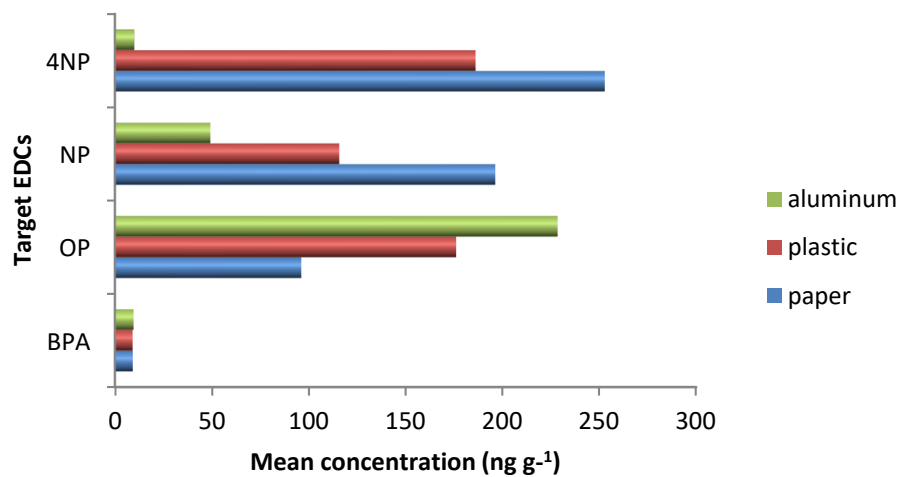


Figure S11. Mean concentrations ($\mu\text{g kg}^{-1}$) of the detected EDCs in relation to the type of the packaging material of the analyzed dairy commodities.