Analysis of chemically labile glycation adducts in seed proteins: a case study of methylglyoxal-derived hydroimidazolone 1 (MG-H1)

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Supplementary information

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Directory

Protocols

Protocol S-1 Solid phase extraction (SPE) procedure for CHROMABOND HR-XAW weak anion exchanger cartridges

Materials:

SPE cartridges: CHROMABOND HR-XA, 200 mg of material, designed for 3 ml load volume

Conditioning solution: methanol (LC-MS grade)

Equilibration solution: water

Eluent 1: 0.1 mol/L NaOH in water (MilliQ)

Eluent 2: methanol (LC-MS grade)

Eluent 3: 1% (v/v) aq. formic acid (LC-MS grade) in methanol (LC-MS grade)

Eluent 4: 5% (v/v) aq. formic acid (LC-MS grade) in methanol (LC-MS grade)

Eluent 5: 10% (v/v) aq. formic acid (LC-MS grade) in methanol (LC-MS grade)

Sample aspiration: the mixture of 146.2 µmol lysine, 1.15 µmol arginine and 1.29 µmol histidine in 1 mL of phosphate buffered saline (PBS) adjusted to pH 10.0 with 25% ammonia solution

Elution – vacuum-drived, 800-900 mbar

Procedure:

#	Step	Applied solution	Volume (mL)
1	Conditioning	Conditioning solution	5
2	Equilibration	Equilibration solution	5
3	Sample aspiration	1 mL of PBS adjusted to pH 10.0	1
4	Elution 1	Eluent 1	5
5	Elution 2	Eluent 2	2
6	Elution 3	Eluent 3	4

7	Elution 4	Eluent 4	4
8	Elution 5	Eluent 5	4

Protocol S-2 Solid phase extraction (SPE) procedure for CHROMABOND HR-XA weak anion exchanger cartridges

Materials:

SPE cartridges: CHROMABOND HR-XA, 200 mg of material, 3 ml load volume

Conditioning solution: methanol (LC-MS grade)

Equilibration solution: water

Eluent 1: methanol (LC-MS grade)

Eluent 2: water (MilliQ)

Eluent 3: 0.1 mol/L NaOH in water (MilliQ)

Eluent 4: methanol (LC-MS grade)

Eluent 5: 1% (v/v) aq. formic acid (LC-MS grade) in methanol (LC-MS grade)

Eluent 6: 5% (v/v) aq. formic acid (LC-MS grade) in methanol (LC-MS grade)

Eluent 7: 10% (v/v) aq. formic acid (LC-MS grade) in methanol (LC-MS grade)

Sample aspiration: the mixture of 146.2 µmol lysine, 1.15 µmol arginine and 1.29 µmol histidine in 1 mL of phosphate buffered saline (PBS) adjusted to pH 10.0 with 25% ammonia

Elution – vacuum-drived, 800-900 mbar

Procedure:

#	Step	Applied solution	Volume (mL)
1	Conditioning	Conditioning solution	5
2	Equilibration	Equilibration solution	5
3	Sample aspiration	1 mL of PBS adjusted to pH 10.0	1
4	Elution 1	Eluent 1	5
5	Elution 2	Eluent 2	5
6	Elution 3	Eluent 3	5
7	Elution 4	Eluent 4	2

8	Elution 5	Eluent 5	4
9	Elution 6	Eluent 6	4
10	Elution 7	Eluent 5	4

Protocol S-3 Solid phase extraction (SPE) procedure for CHROMABOND C18 reversed phase (RP) cartridges

Materials:

SPE cartridges: CHROMABOND C18 reversed phase (RP), 200 mg of material, 3 ml load

Conditioning solution: methanol (LC-MS grade)

Equilibration solution: water

Eluent 1: methanol (LC-MS grade)

Eluent 2: water (MilliQ)

Eluent 3: 0.25 mol/L ammonium acetate (LC-MS grade) in water (MilliQ)

Eluent 4: 0.1 mol/L ammonia (LC-MS grade) in water (MilliQ)

Eluent 5: 1% (v/v) ammonia (LC-MS grade), aq. 10 % (v/v) acetonitrile (LC-MS grade)

Eluent 6: 1% (v/v) ammonia (LC-MS grade), aq. 20 % (v/v) acetonitrile (LC-MS grade)

Eluent 7: 1% (v/v) ammonia (LC-MS grade), aq. 40 % (v/v) acetonitrile (LC-MS grade)

Sample aspiration: the mixture of 146.2 µmol lysine, 1.15 µmol arginine and 1.29 µmol histidine in 1 mL of phosphate buffered saline (PBS) adjusted to pH 10.0 with 25% ammonia

Elution – vacuum-drived, 800-900 mbar

Procedure:

#	Step	Applied solution	Volume (mL)
1	Conditioning	Conditioning solution	5
2	Equilibration	Equilibration solution	5
3	Sample aspiration	1 mL of PBS adjusted to pH 10.0	1
4	Elution 1	Eluent 1	5
5	Elution 2	Eluent 2	5
6	Elution 3	Eluent 3	5

7	Elution 4	Eluent 4	5
8	Elution 5	Eluent 5	4
9	Elution 6	Eluent 6	4
10	Elution 7	Eluent 5	4

Tables

Table S-1 Protein recoveries and total UV densities calculated for individual pea samples separated by SDS-PAGE

Sample	Sample weight (mg)	Protein concentration (mg/mL)	Protein recovery (mg/g fresh weight)	UV densities (AU)[a]
Pea-1	49.5	38.1	96.2	38200
Pea-2	50.4	39.3	97.5	36200
Pea-3	51.6	42.4	102.8	34100
Pea-AA-5d-1	51.4	32.8	87.8	34100
Pea-AA-5d-2	50.3	54.9	129.8	35500
Pea-AA-5d-3	50.3	35.1	91.6	38000

Pea and Pea-AA-5d denote the seeds of yellow-seeded cultivar Millennium before and after accelerated ageing (AA) during five days, respectively; AU, arbitrary units

Table S-2 Protein recoveries and total UV densities calculated for individual oilseed rape samples separated by SDS-PAGE

Sample	Sample weight (mg)	Protein concentration (mg/mL)	Protein recovery (mg/g fresh weight)	UV densities (AU)[a]
Brassica-1	209.4	73,93	103,50	29315
Brassica-2	204.7	77,34	108,28	28814
Brassica-3	213.1	70,42	98,59	28361
Brassica-4	209.7	68,64	96,10	28075
Brassica-5	210.2	85,31	119,44	28361
Brassica-NA-1	211.5	58,64	82,10	28326
Brassica-NA-2	212.9	38,50	53,90	28837
Brassica-NA-3	203.6	58,67	82,14	29695
Brassica-NA-4	207.3	67,46	94,44	30906
Brassica-NA-5	207.2	80,31	112,44	30126

Brassica and Brassica-NA denote the seeds of the oilseed rape cultivar Oredezh-2 (K-4917) after one and nine years of natural ageing (dark, 18° C), respectively; AU, arbitrary units

Table S-3 Instrument settings applied for Orbitrap-LIT-MS and MS/MS experiments

Parameter	Setting
MS conditions	
Ionization mode	Positive
Mass analyzer	LIT-Orbitrap (FT-scan)
Ion spray voltage (IS)	4.0 kV
Nebulizer gas	35 psig
Auxillary gas	30 psig
Capillary temperature	275 °C
Mass to charge ratio (*m/z*) range	400 – 2000
Resolution	30000
MS/MS conditions	
Ionization mode	Positive
Mass analyzer	LIT-Orbitrap (FT-scan)
Ion spray voltage (IS)	4.0 kV
Fragmentation	Collision activated dissociation
Isolation width	2 Da
Charge state rejected	1+
Normalized collision energy	35%
Activation frequency	0.25
Activation time	10 ms

Figures

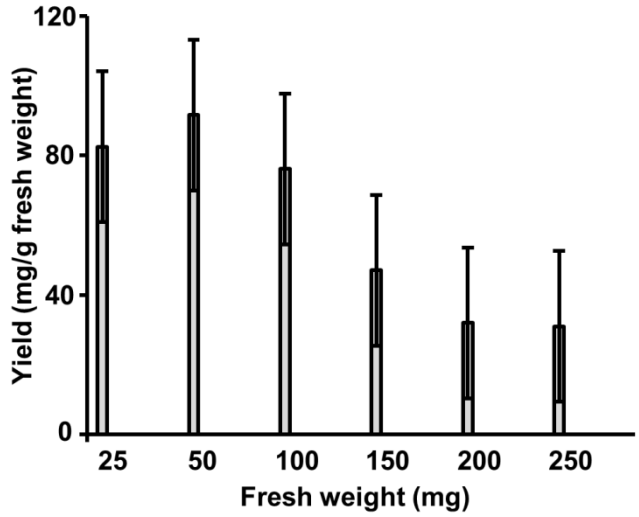
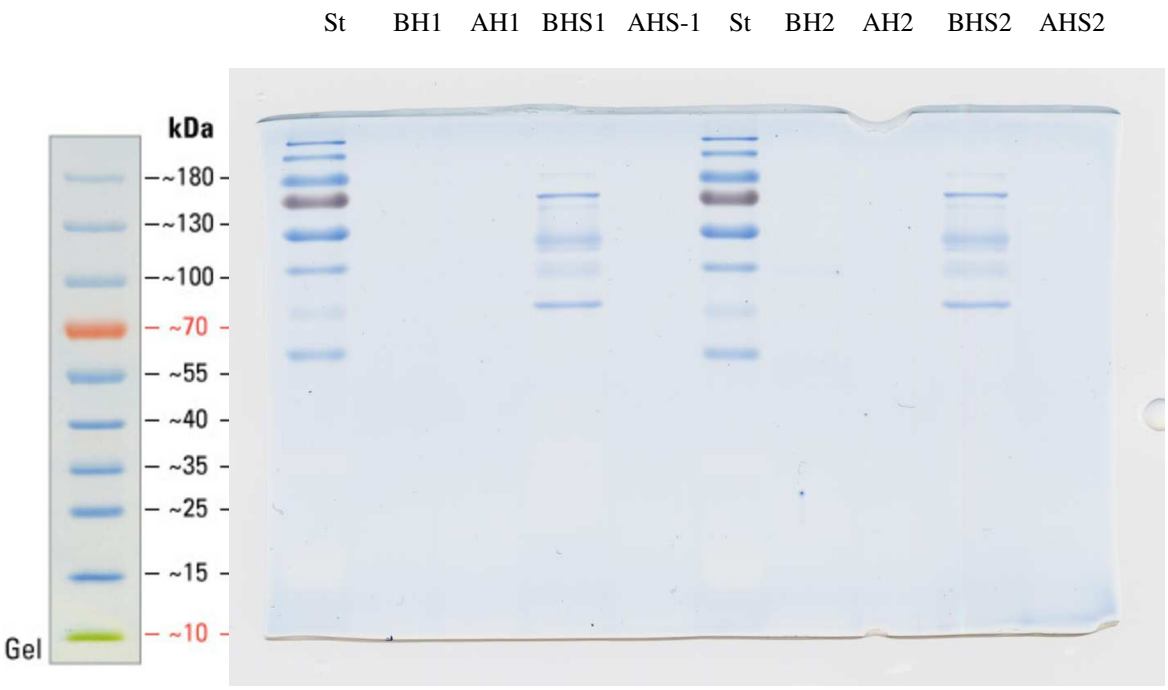


Figure S-1 Optimization of protein yield in respect of seed material amounts, taken for phenol extraction

A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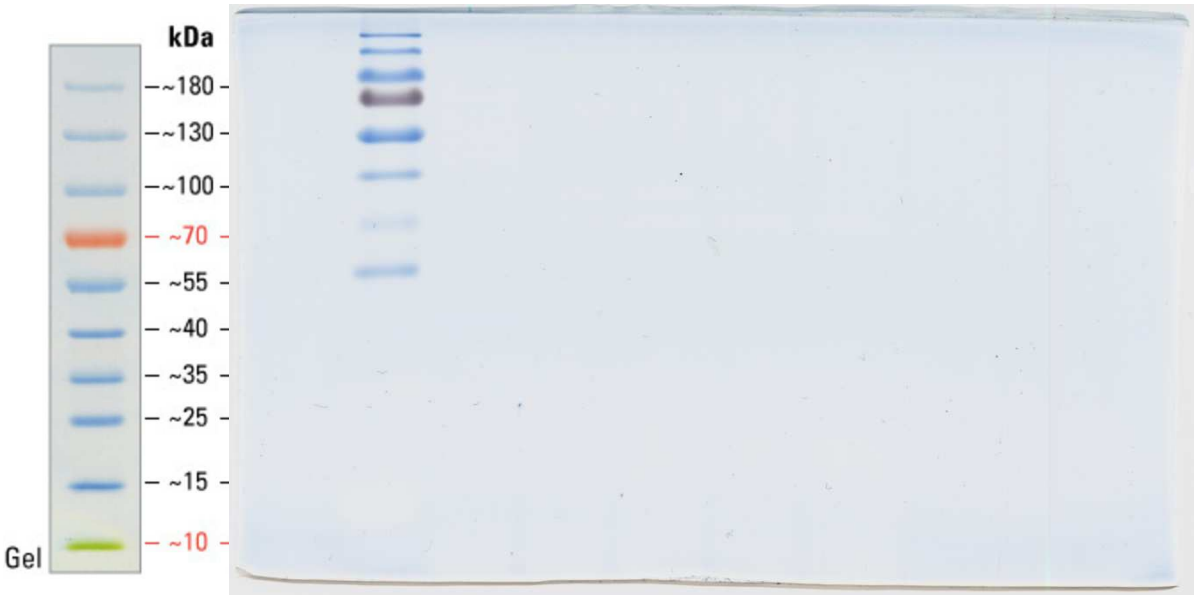
~100
~70
~55
~40
~35
~25
~15
~10
Gel

B


St BH1 APE1 APES1 APK-1 AH1
kDa
~180
~130
~100
~70
~55
~40
~35
~25
~15
~10
Gel

C

Figure S-2 SDS-PAGE electropherograms of glycated bovine serum albumin (BSA), before and after individual steps of enzymatic hydrolysis. Hydrolysis was performed in triplicates. The aliquots (5 µg) of all samples were loaded on a gel in 10 µL of sample buffer. BH, before hydrolysis; APE, after incubation with Pronase E; APK, after incubation with Proteinase K; AH, after complete hydrolysis; BHS, before hydrolysis in presence of 0.5% (w/v) SDS; APES, after incubation with Pronase E in presence of 0.5% (w/v) SDS; APK, after incubation with Proteinase K in presence of 0.5% (w/v) SDS; AH, after complete hydrolysis in presence of 0.5% (w/v) SDS; St, Page Ruler Prestained Protein Ladder

A

St BH1 AH1 BHS1 AHS-1 St BH2 AH2 BHS2 AHS2

B

St BH1 APE1 APE1 APK-1 AH-1 BH-3AH3BH4AH4

C

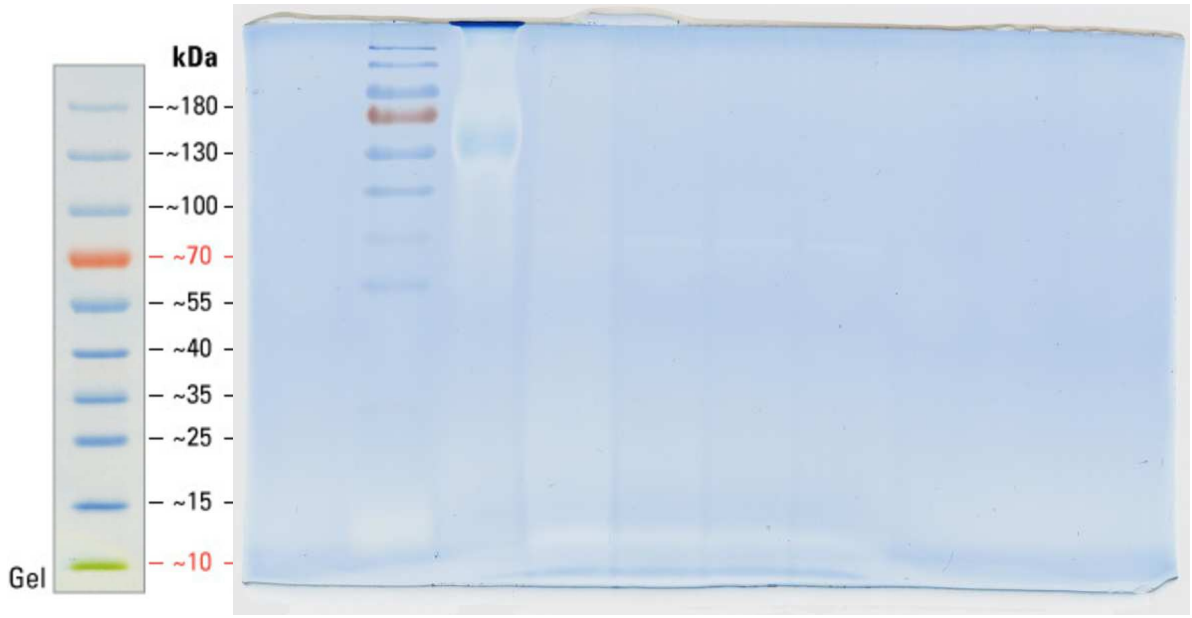


Figure S-3 SDS-PAGE electropherograms of glycated bovine serum albumin, before and after individual steps of enzymatic hydrolysis. Hydrolysis was performed in triplicates. The aliquots (5 µg) of all samples were loaded on a gel in 10 µL of sample buffer. BH, before hydrolysis; APE, after incubation with Pronase E; APK, after incubation with Proteinase K; AH, after complete hydrolysis; BHS, before hydrolysis in presence of 0.5% (w/v) SDS; APES, after incubation with Pronase E in presence of 0.5% (w/v) SDS; APK, after incubation with Proteinase K in presence of 0.5% (w/v) SDS; AH, after complete hydrolysis in presence of 0.5% (w/v) SDS; St, Page Ruler Prestained Protein Ladder

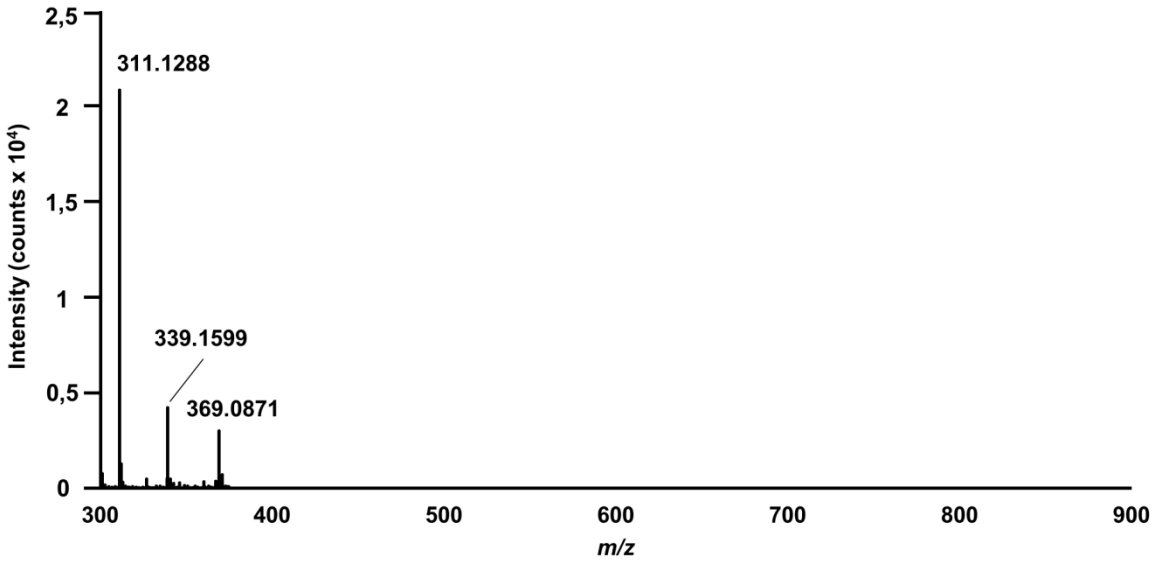


Figure S-4 ESI-QqTOF mass spectrum, obtained for enzymatic hydrolysate of pea protein. The spectrum was acquired by a syringe infusion (10 µL/min) in a QqTOF-MS (Triple TOF, Sciex, Darmstadt, Germany), operated in positive ion mode. No signals of multiply charged ions could be observed in the spectrum that indicated completeness of hydrolysis.

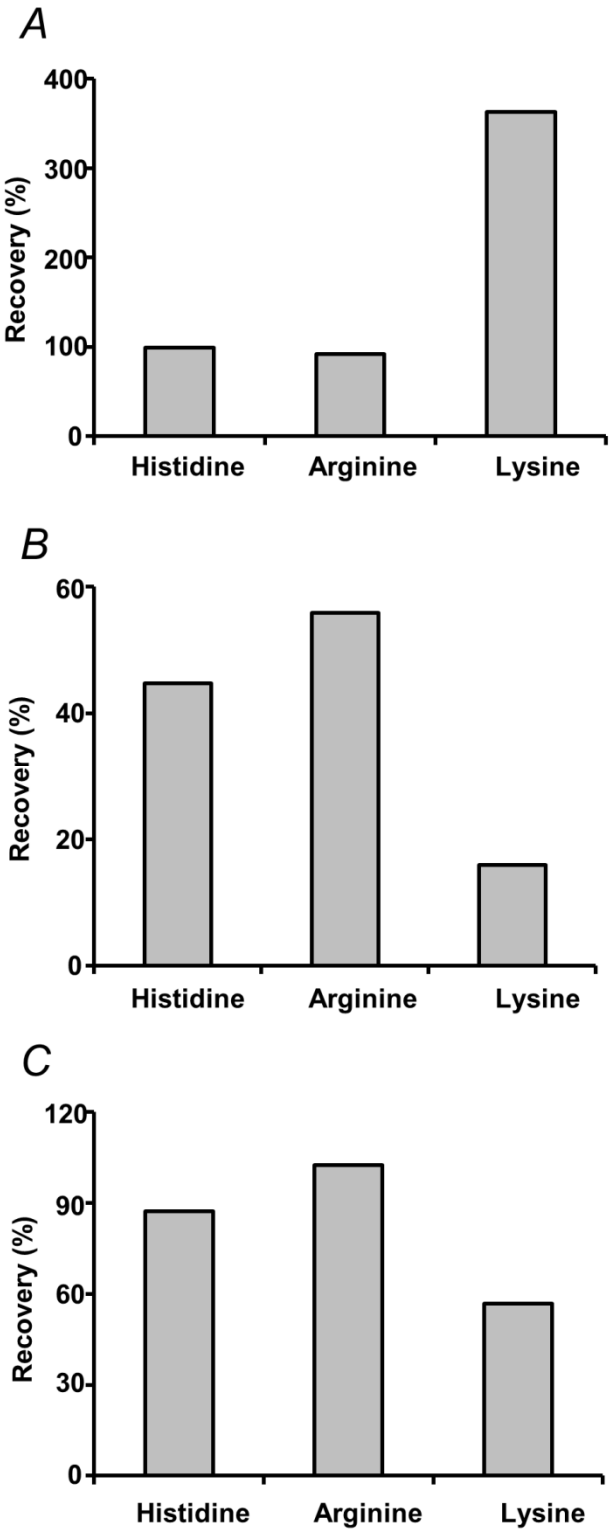


Figure S-5 Recovery of three basic amino acids from weak anion exchanger CHROMABOND HR-XAW (A), strong anion exchanger CHROMABOND HR-XA (B) and reversed phase CHROMABOND C18 (C) cartridges (according the Protocols S1-1, 2 and 3)

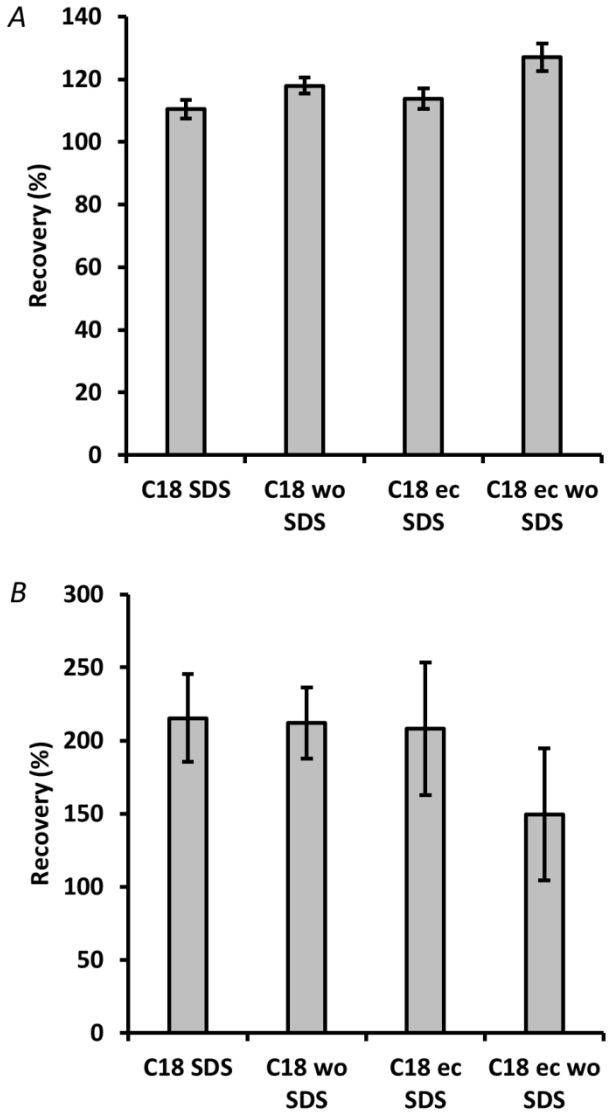


Figure S-6 Recovery (%) of N^{ε}-(carboxymethyl)lysine (CML, A) and lysine (B) from enzymatic hydrolysates obtained with 1 mg/mL glycated BSA in presence and absence of 0.5% (w/v) SDS with subsequent SPE on CHROMABOND C18 and C18 ec cartridges. Relative abundances of analytes were obtained by integration of corresponding LC-MS extracted ion chromatograms (XICs) at *m/z* 485.20 and 427.19 for the L-FDVA derivatives of CML and lysine, respectively, and related to abundances observed without application of SDS and SPE.