SUPPORTING INFORMATION

Association of enzymatically and non-enzymatically functionalized caseins analyzed by size-exclusion chromatography and light scattering techniques

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Quantitation of Maillard Reaction Products by High-Pressure Liquid Chromatography with Tandem Mass Spectrometric Detection (HPLC-MS/MS). Protein-bound Maillard reaction products were quantitated in enzymatic hydrolyzed casein samples by HPLC-MS/MS with standard addition. Enzymatic hydrolysis and HPLC-MS/MS were performed according to Hellwig et al.[1] All samples were filtrated (0.45 µm, regenerated cellulose) before injection. The added standard solutions contained the target analytes in the following concentrations: 1.0 µg/mL N-ε-carboxyethyllysine (CEL), 2.0 µg/mL formyline, 0.5 µg/mL methylglyoxal-derived hydroimidazolone 1 (MG-H1), and 2.0 µg/mL pyrraline 2.0 µg/mL for control and glycated samples and 19.9 µg/mL CEL, 0.4 µg/mL formyline, 5.0 µg/mL MG-H1, and 2.0 µg/mL pyrraline for MGO-modified sample, respectively. N-ε-carboxymethyllysine (CML) was quantified after acid hydrolysis and reduction by HPLC-MS/MS with external calibration and an internal standard (D_2-CML) according to Moeckel et al.[2]

Lipid Extraction and Identification by Thin-layer Chromatography (TLC).

Lipids were extracted from casein lyophilizates using the Folch method[3,4] and separated by TLC according to Helmerich & Koehler[5]. Therefore, 500 µL ice-cold methanol followed by 1000 µL ice-cold chloroform were added to 10 mg casein, vortex-homogenized for 30 s and incubated for 20 min at -20 °C. After centrifugation at 2.000×g for 20 min, the supernatant was filtrated (0.45 µm, regenerated cellulose) and an aliquot of 1000 µL was evaporated under nitrogen stream. The dried lipid extracts were resolubilized in 200 µL n-hexane:2-propanol (4:1, v/v). 10 mg of a freeze-dried sample of commercial butter milk was extracted analogously and solubilized in 500 µL n-hexane:2-propanol (4:1, v/v). Soy lecithin, sunflower oil, oleic acid, and cholesterol

dissolved to 0.5 mg/mL in n-hexane:2-propanol (4:1, v/v), respectively, were used as standards for TLC. 12 µL of sample extracts and 6 µL of standards were applied band-wise (5 mm) onto a HPTLC plate (silica G 60, 200×100×0.25 mm, Merck) using a glass capillary (2 µL) and developed with methyl acetate/chloroform/1-propanol/methanol/0.25% aqueous potassium chloride (25:25:25:10:9, v/v/v/v/v). Plate was dipped into an 3.3% (w/v) copper sulfate solution containing 14% (w/v) phosphoric acid, heated at 180 °C for 10 min and visualized afterward under white light.

Table S1. Concentrations of individual Maillard reaction products given in µmol/g protein (corresponding percentage of lysine/arginine modification in parentheses) of untreated casein (control), after glycation with lactose for 36 h (Lac-36) and 48 h (Lac-48) and upon modification with MGO as well as performance parameters of the LC MS/MS analysis. Values expressed as mean ± standard deviation (n=2).

Analyt	control	Lac-36	Lac-48	MGO	LOD[a]	LOQ[a]
Amadori products[b]	9.1 ± 0.2 (2.0)	241.8 ± 1.3 (40.8)	245.6 ± 15.5 (42.2)	-	-	-
CML	0.7 ± 0.0 (0.1)	41.0 ± 1.5 (7.5)	47.5 ± 1.2 (8.7)	-	0.07	0.20
pyrraline	tr	3.3 ± 0.3 (0.6)	3.5 ± 0.1 (0.6)	tr	0.04	0.13
CEL	n.d.	0.31 ± 0.03 (< 0.1)	0.35 ± 0.07 (< 0.1)	24.8 ± 0.7 (4.4)	0.08	0.25
formyline	n.d.	0.19 ± 0.01 (< 0.1)	0.18 ± 0.03 (< 0.1)	n.d.	0.02	0.06
MG-H1	n.d.	tr	0.17 ± 0.03 (< 0.1)	27.3 ± 3.0 (11.6)	0.05	0.14

n.d., not detected (below LOD); tr, trace amounts (between LOD and LOQ)

[a] LOD and LOQ based on an amount of 3 mg protein per enzymatic hydrolysis

[b] data from Hannß et al.[6]

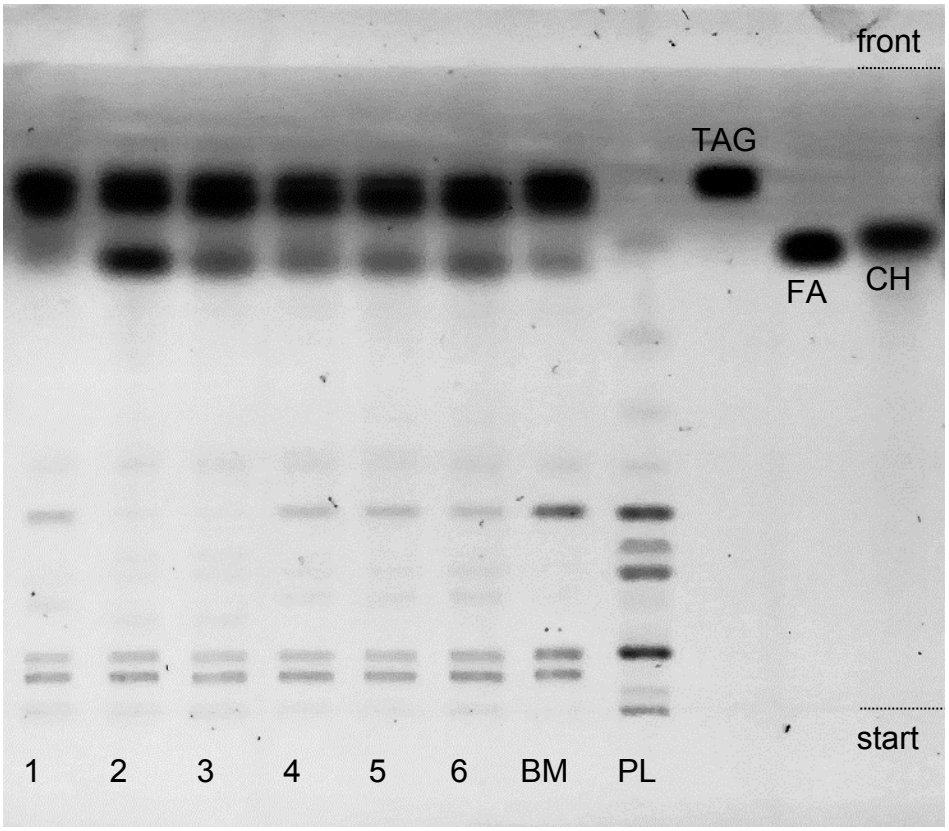

Figure S1. Separation of lipid components by TLC after FOLCH-extraction, developed with methyl acetate/chloroform/1-propanol/methanol/0.25% potassium chloride (25:25:25:10:9, v/v/v/v/v) and detected with 3.3% (w/v) copper sulfate in 14% (w/v) phosphoric acid; applied casein samples: 1 = control, 2 = glycated with lactose (36 h), 3 = glycated with lactose (48 h), 4 = cross-linked with MGO, 5 = cross-linked with mTG, 6 = cross-linked with GTA, applied standards: *BM* = lipids extracted from butter milk; *PL* = phospholipids from soy lecithin; *TAG* = triacylglycerides from sunflower oil; *FA* = free fatty acids (oleic acid); *CH* = cholesterol; Applied sample volume for 1-6: 12 µL, standards: 6 µL.

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