

Supplementary Information - The effect of pharmaceutical excipients on protein chemical degradation through deamidation and isomerization

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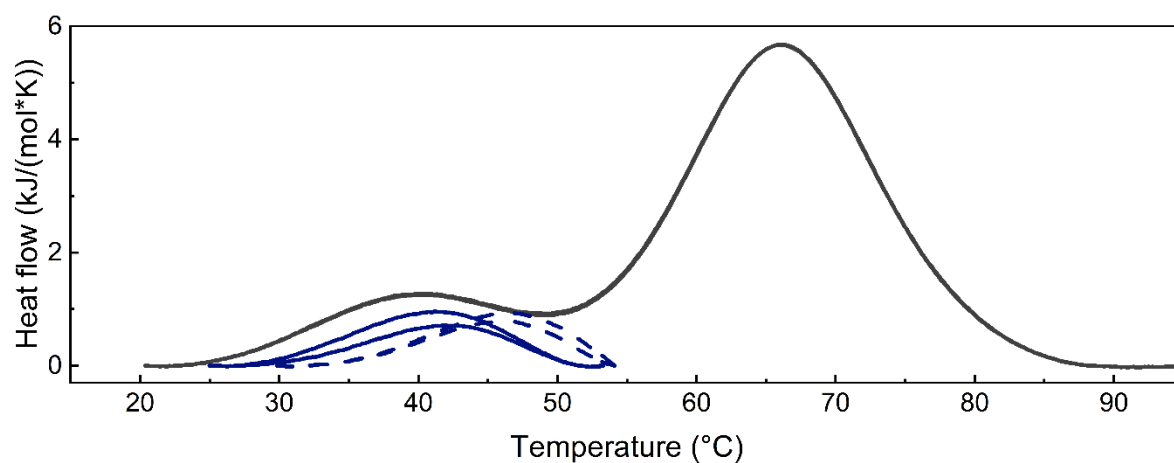


Fig. S1 DSC thermograms of GA-Z in 0 (—), 10 (—), and 20 (---) % v/v fructose. All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0). Measurements were performed in duplicate, and the data are included in the figure

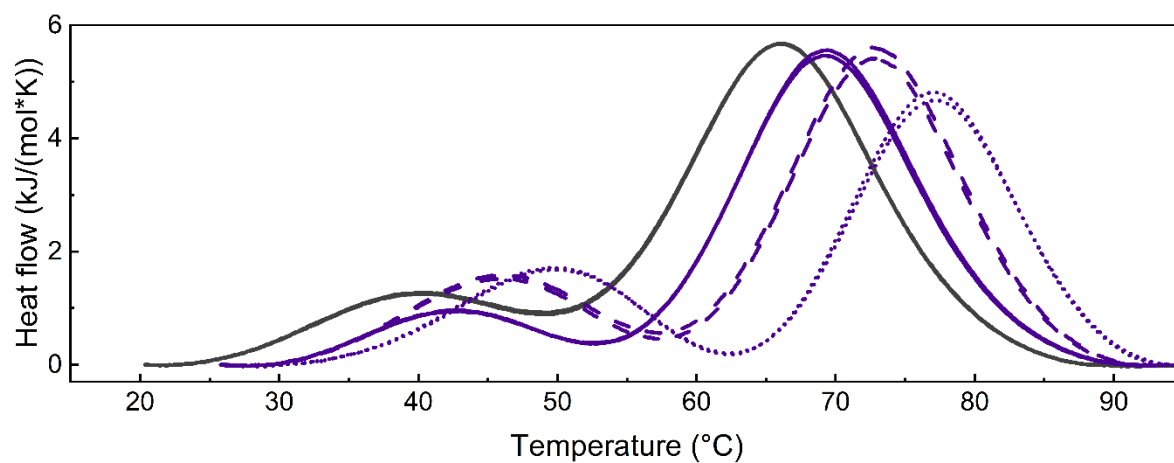


Fig. S2 DSC thermograms of GA-Z in 0 (—), 10 (—), 20 (---), and 30 (···) % v/v sucrose. All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0). Measurements were performed in duplicate, and the data are included in the figure

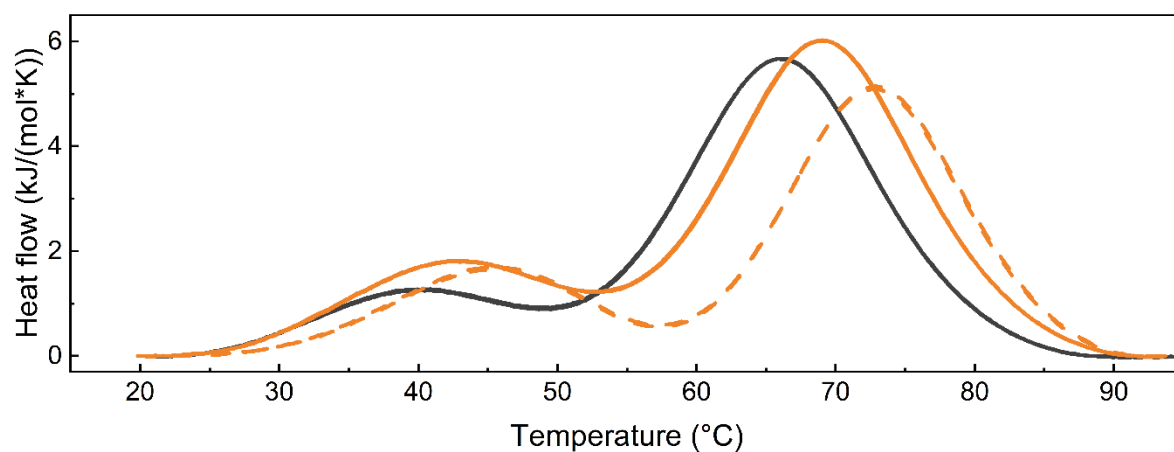


Fig. S3 DSC thermograms of GA-Z in 0 (—), 10 (—), and 20 (---) % v/v melezitose. All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0). Measurements were performed in duplicate, and the data are included in the figure

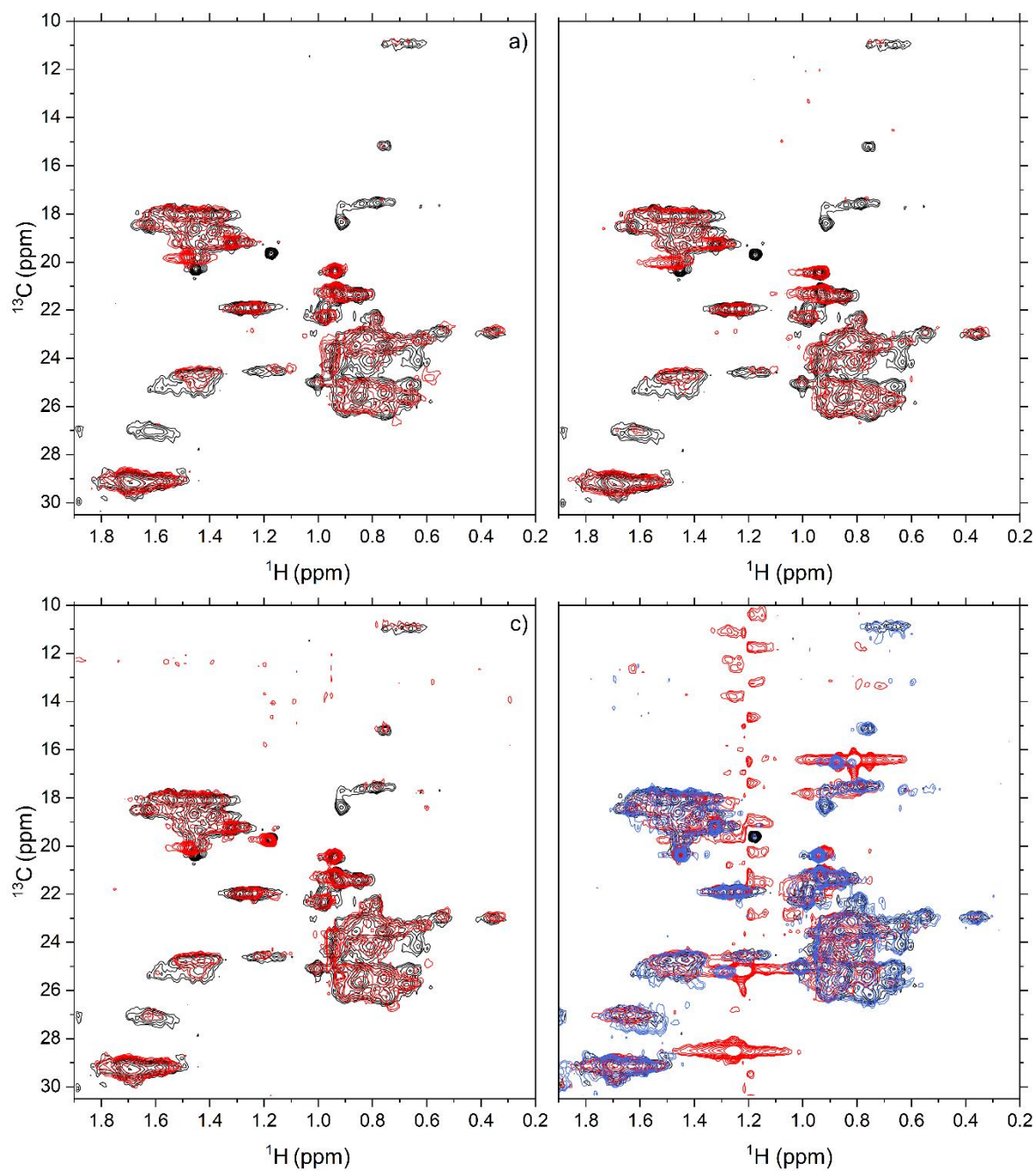


Fig. S4 HMQC spectra of GA-Z in a) 0 (—) and 20 (—) % v/v fructose, b) 0 (—) and 20 (—) % v/v sucrose, c) 0 (—) and 20 (—) % v/v melezitose, and d) 0 mM surfactant (—), 10.9 mM DDM (—), and 0.8 mM polysorbate 80 (—). All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0), and the spectra were collected at 37°C

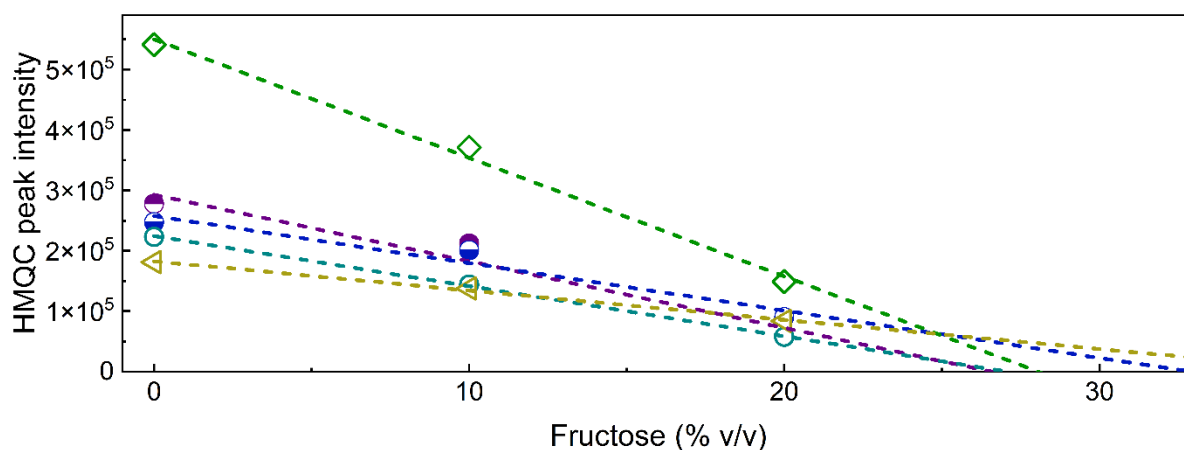


Fig. S5 HMQC peak intensities of the GA-Z residues Ile16^(Cγ2-Hγ2) (●), Leu28^(Cγ-Hγ) (●), Leu34^(Cγ-Hγ) (○), Glu47^(Cβ-Hβ) (◇), and Lys49^(Cγ-Hγ) (◁) in 0-20% v/v fructose analyzed at 37°C. The slope equations for the residues are $I = -11013 \cdot X + 292803$ (---), $I = -7835 \cdot X + 257891$ (---), $I = -8286.9 \cdot X + 224533$ (---), $I = -19609 \cdot X + 550046$ (---), and $I = -4839.3 \cdot X + 182757$ (---). All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0)

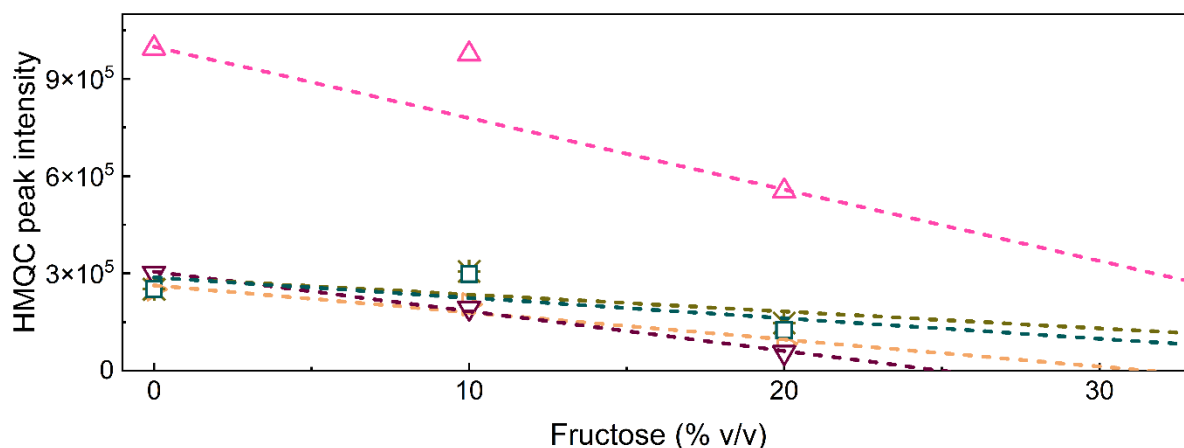


Fig. S6 HMQC peak intensities of the GA-Z residues Leu74^(Cδ2-Hδ2) (▷), Lys84^(Cδ-Hδ) (△), Ile103^(Cγ2-Hγ2) (▽), Leu104^(Cδ2-Hδ2) (*), and Leu107^(Cδ2-Hδ2) (□) in 0-20% v/v fructose analyzed at 37°C. The slope equations for the residues are $I = -8363.6 \cdot X + 263518$ (---), $I = -22043 \cdot X + 1000000$ (---), $I = -12242 \cdot X + 305975$ (---), $I = 5219.4 \cdot X + 287160$ (---), and $I = -6307.2 \cdot X + 288019$ (---). All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0)

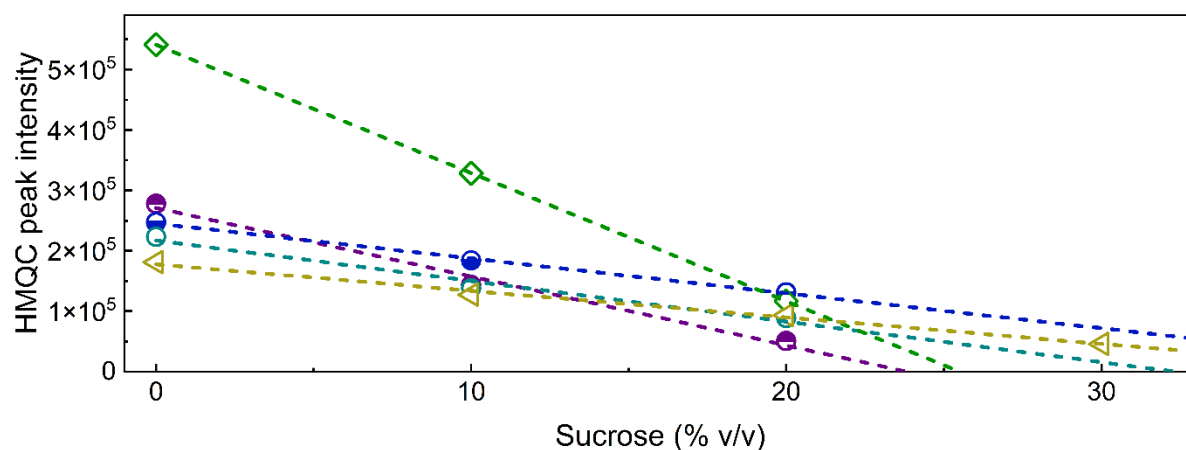


Fig. S7 HMQC peak intensities of the GA-Z residues Ile16^(C γ 2-H γ 2) (●), Leu28^(C γ -H γ) (●), Leu34^(C γ -H γ) (○), Glu47^(C β -H β) (◇), and Lys49^(C γ -H γ) (◁) in 0-30% v/v sucrose analyzed at 37°C. The slope equations for the residues are $I = -11372 \cdot X + 271132$ (---), $I = -5783.9 \cdot X + 245385$ (---), $I = -6717.5 \cdot X + 217275$ (---), $I = -21214 \cdot X + 540984$ (---), and $I = -4386.7 \cdot X + 177756$ (---). All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0)

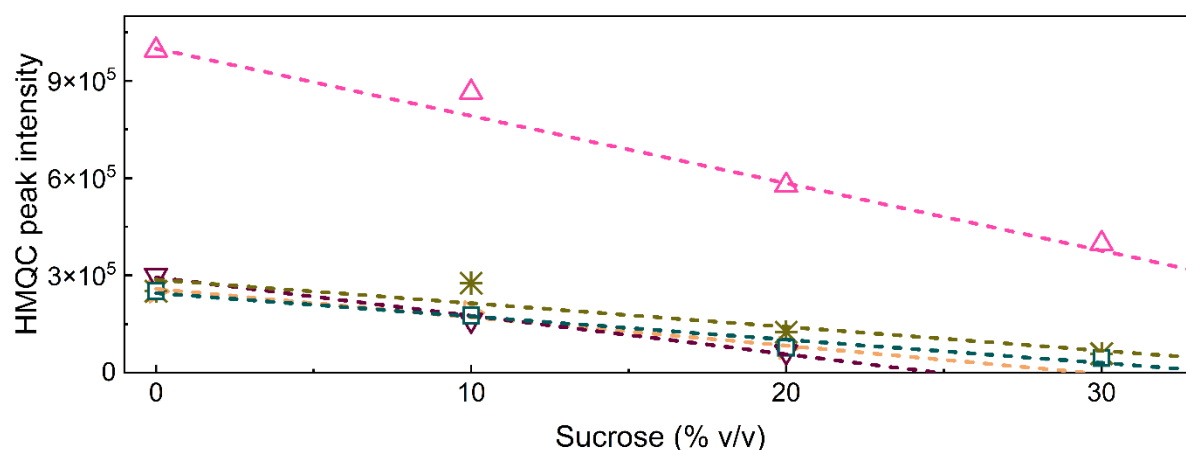


Fig. S8 HMQC peak intensities of the GA-Z residues Leu74^(C δ 2-H δ 2) (▷), Lys84^(C δ -H δ) (△), Ile103^(C γ 2-H γ 2) (▽), Leu104^(C δ 2-H δ 2) (*), and Leu107^(C δ 2-H δ 2) (◻) in 0-30% v/v sucrose analyzed at 37°C. The slope equations for the residues are $I = -8752.9 \cdot X + 258537$ (---), $I = -20770 \cdot X + 1000000$ (---), $I = -11845 \cdot X + 294049$ (---), $I = -7296.5 \cdot X + 287314$ (---), and $I = -7175 \cdot X + 245456$ (---). All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0)

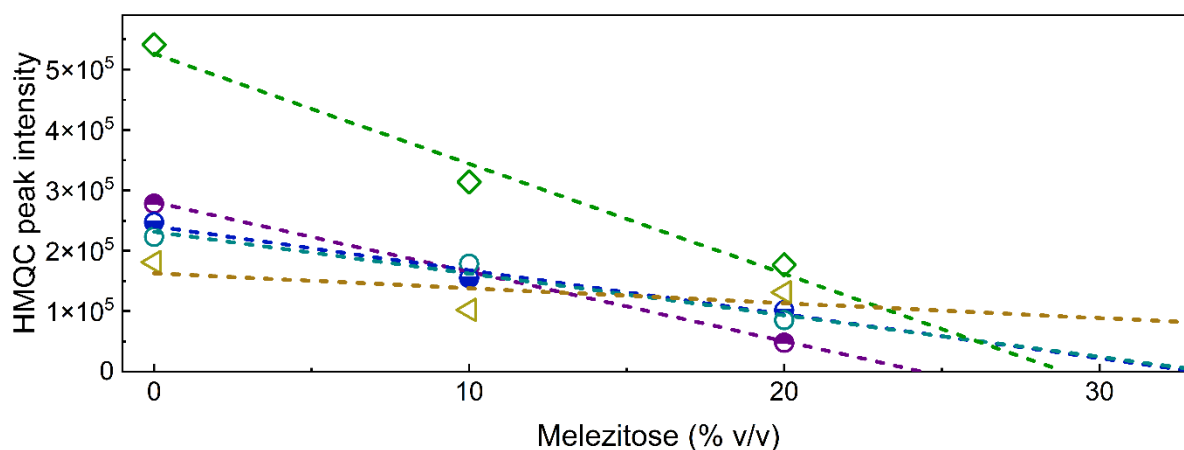


Fig. S9 HMQC peak intensities of the GA-Z residues Ile16^(C γ 2-H γ 2) (●), Leu28^(C γ -H γ) (●), Leu34^(C γ -H γ) (○), Glu47^(C β -H β) (◇), and Lys49^(C γ -H γ) (◁) in 0-30% v/v sucrose analyzed at 37°C. The slope equations for the residues are $I = -11504 \cdot X + 280528$ (---), $I = -7281.9 \cdot X + 240772$ (---), $I = 6901.1 \cdot X + 231509$ (---), $I = -18220 \cdot X + 526229$ (---), and $I = -2470.5 \cdot X + 162949$ (---). All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0)

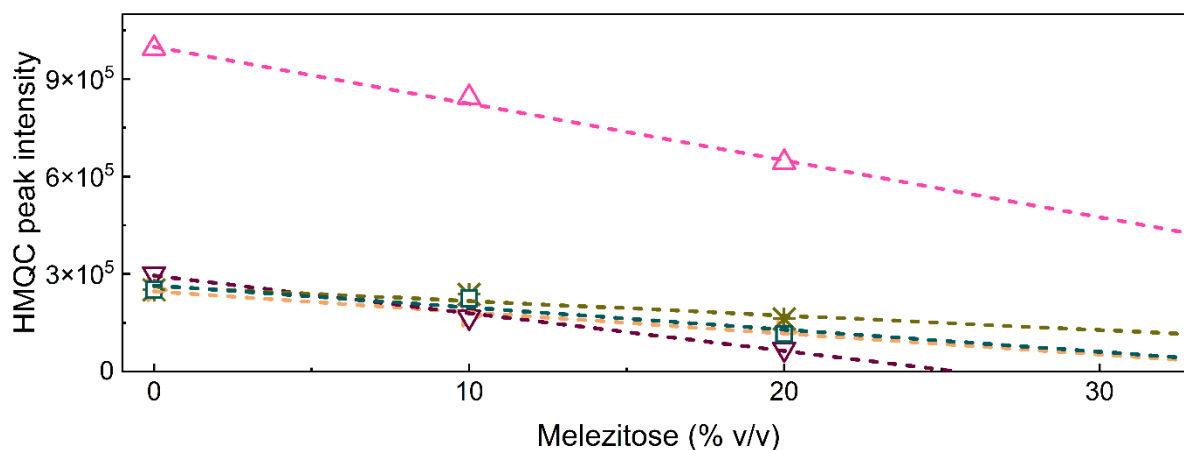


Fig. S10 HMQC peak intensities of the GA-Z residues Leu74^(C δ 2-H δ 2) (▷), Lys84^(C δ -H δ) (△), Ile103^(C γ 2-H γ 2) (▽), Leu104^(C δ 2-H δ 2) (*), and Leu107^(C δ 2-H δ 2) (◻) in 0-30% v/v sucrose analyzed at 37°C. The slope equations for the residues are $I = -6489.9 \cdot X + 246995$ (---), $I = -17509 \cdot X + 1000000$ (---), $I = -11583 \cdot X + 295216$ (---), $I = -4470.1 \cdot X + 261794$ (---), and $I = -6809.8 \cdot X + 265276$ (---). All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0)

Table S1. HMQC peak shifts of GA-Z residues in 0-30% v/v fructose, sucrose, and melezitose analyzed at 37°C. All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0)

	0% v/v	10% v/v	20% v/v	30% v/v
Fructose				
Ile16- ¹³ Cγ2 (ppm)	18.385	18.398	18.274	
Ile16- ¹ Hγ2 (ppm)	0.918	0.917	0.910	
Leu28- ¹³ Cγ (ppm)	27.448	27.427	27.480	
Leu28- ¹ Hγ (ppm)	2.039	2.040	2.053	
Leu34- ¹³ Cγ (ppm)	27.081	27.049	26.809	
Leu34- ¹ Hγ (ppm)	1.611	1.610	1.592	
Glu47- ¹³ Cβ (ppm)	29.649	29.542	29.497	
Glu47- ¹ Hβ (ppm)	2.082	2.084	2.068	
Lys49- ¹³ Cγ (ppm)	24.588	24.587	24.444	
Lys49- ¹ Hγ (ppm)	1.226	1.206	1.164	
Leu74- ¹³ Cδ2 (ppm)	22.945	22.848	22.787	
Leu74- ¹ Hδ2 (ppm)	0.552	0.550	0.528	
Lys84- ¹³ Cδ (ppm)	29.163	29.076	29.024	
Lys84- ¹ Hδ (ppm)	1.703	1.707	1.703	
Ile103- ¹³ Cγ2 (ppm)	17.656	17.509	17.449	
Ile103- ¹ Hγ2 (ppm)	0.785	0.777	0.770	
Leu104- ¹³ Cδ2 (ppm)	22.991	22.956	22.887	
Leu104- ¹ Hδ2 (ppm)	0.361	0.353	0.349	
Leu107- ¹³ Cδ2 (ppm)	23.342	23.321	23.255	
Leu107- ¹ Hδ2 (ppm)	0.637	0.622	0.615	
Sucrose				
Ile16- ¹³ Cγ2 (ppm)	18.385	18.410	18.035	
Ile16- ¹ Hγ2 (ppm)	0.918	0.915	0.891	
Leu28- ¹³ Cγ (ppm)	27.448	27.479	27.474	
Leu28- ¹ Hγ (ppm)	2.039	2.048	2.051	
Leu34- ¹³ Cγ (ppm)	27.081	27.022	27.004	
Leu34- ¹ Hγ (ppm)	1.611	1.612	1.617	
Glu47- ¹³ Cβ (ppm)	29.649	29.549	29.464	
Glu47- ¹ Hβ (ppm)	2.082	2.075	2.082	
Lys49- ¹³ Cγ (ppm)	24.588	24.547	24.500	24.459

Lys49- ¹ H γ (ppm)	1.226	1.201	1.190	1.169
Leu74- ¹³ C δ 2 (ppm)	22.945	22.856	22.907	
Leu74- ¹ H δ 2 (ppm)	0.552	0.547	0.554	
Lys84- ¹³ C δ (ppm)	29.163	29.128	29.069	28.942
Lys84- ¹ H δ (ppm)	1.703	1.699	1.696	1.702
Ile103- ¹³ C γ 2 (ppm)	17.656	17.477	17.345	
Ile103- ¹ H γ 2 (ppm)	0.785	0.785	0.768	
Leu104- ¹³ C δ 2 (ppm)	22.991	22.966	22.871	22.866
Leu104- ¹ H δ 2 (ppm)	0.361	0.354	0.350	0.335
Leu107- ¹³ C δ 2 (ppm)	23.342	23.326	23.292	23.277
Leu107- ¹ H δ 2 (ppm)	0.637	0.621	0.611	0.597

Melezitose

Ile16- ¹³ C γ 2 (ppm)	18.385	18.398	18.38	
Ile16- ¹ H γ 2 (ppm)	0.918	0.921	0.918	
Leu28- ¹³ C γ (ppm)	27.448	27.461	27.386	
Leu28- ¹ H γ (ppm)	2.039	2.033	2.031	
Leu34- ¹³ C γ (ppm)	27.081	27.034	26.986	
Leu34- ¹ H γ (ppm)	1.611	1.630	1.624	
Glu47- ¹³ C β (ppm)	29.649	29.574	29.517	
Glu47- ¹ H β (ppm)	2.082	2.084	2.093	
Lys49- ¹³ C γ (ppm)	24.588	24.58	24.509	
Lys49- ¹ H γ (ppm)	1.226	1.200	1.197	
Leu74- ¹³ C δ 2 (ppm)	22.945	22.944	22.827	
Leu74- ¹ H δ 2 (ppm)	0.552	0.553	0.541	
Lys84- ¹³ C δ (ppm)	29.163	29.145	29.055	
Lys84- ¹ H δ (ppm)	1.703	1.701	1.706	
Ile103- ¹³ C γ 2 (ppm)	17.656	17.572	17.42	
Ile103- ¹ H γ 2 (ppm)	0.785	0.788	0.794	
Leu104- ¹³ C δ 2 (ppm)	22.991	22.997	22.944	
Leu104- ¹ H δ 2 (ppm)	0.361	0.359	0.357	
Leu107- ¹³ C δ 2 (ppm)	23.342	23.329	23.288	
Leu107- ¹ H δ 2 (ppm)	0.637	0.635	0.614	

Table S2. HMQC peak shifts of GA-Z residues in 0-10.9 mM DDM and 0-0.8 mM polysorbate 80 analyzed at 37°C. All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0)

DDM	0 mM	1.1 mM	5.5 mM	10.9 mM
Lys4- ¹³ Cδ (ppm)	29.184	29.202	29.188	29.169
Lys4- ¹ Hδ (ppm)	1.586	1.587	1.597	1.601
Ile16- ¹³ Cγ2 (ppm)	18.385	18.407	18.482	
Ile16- ¹ Hγ2 (ppm)	0.918	0.913	0.925	
Leu19- ¹³ Cδ2 (ppm)	24.147	23.976		
Leu19- ¹ Hδ2 (ppm)	0.636	0.667		
Thr23- ¹³ Cδ (ppm)	21.978	22.010	21.990	21.982
Thr23- ¹³ Hδ2 (ppm)	1.290	1.281	1.274	1.267
Leu28- ¹³ Cγ (ppm)	27.448	27.515	27.487	27.434
Leu28- ¹ Hγ (ppm)	2.039	2.042	2.036	2.027
Leu34- ¹³ Cγ (ppm)	27.081	27.105	27.082	27.066
Leu34- ¹ Hγ (ppm)	1.611	1.632	1.636	1.638
Glu47- ¹³ Cβ (ppm)	29.649	29.660	29.711	29.899
Glu47- ¹ Hβ (ppm)	2.082	2.070	2.069	2.070
Lys49- ¹³ Cγ (ppm)	24.588	24.638	24.630	
Lys49- ¹ Hγ (ppm)	1.226	1.226	1.208	
Leu74- ¹³ Cδ2 (ppm)	22.945	23.044	22.973	22.951
Leu74- ¹ Hδ2 (ppm)	0.552	0.568	0.576	0.546
Lys84- ¹³ Cδ (ppm)	29.163	29.628	29.230	29.218
Lys84- ¹ Hδ (ppm)	1.703	1.690	1.690	1.688
Lys91- ¹³ Cδ (ppm)	24.606	24.657	24.682	
Lys91- ¹ Hδ (ppm)	1.143	1.157	1.165	
Thr92- ¹³ Cδ (ppm)	21.940	22.010	21.935	21.940
Thr92- ¹³ Hδ2 (ppm)	1.235	1.281	1.235	1.236
Ile103- ¹³ Cγ2 (ppm)	17.656	17.619	17.683	17.681
Ile103- ¹ Hγ2 (ppm)	0.785	0.780	0.796	0.801
Leu104- ¹³ Cδ2 (ppm)	22.991	22.998	22.987	22.956
Leu104- ¹ Hδ2 (ppm)	0.361	0.364	0.364	0.367
Leu107- ¹³ Cδ2 (ppm)	23.342	23.407	23.138	23.383
Leu107- ¹ Hδ2 (ppm)	0.637	0.641	0.647	0.626
Polysorbate 80	0 mM	0.1 mM	0.4 mM	0.8 mM
Lys4- ¹³ Cδ (ppm)	29.184	29.203	29.204	29.194

Lys4- ¹ Hδ (ppm)	1.586	1.586	1.585	1.586
Ile16- ¹³ Cγ2 (ppm)	18.385	18.408	18.398	18.407
Ile16- ¹ Hγ2 (ppm)	0.918	0.920	0.919	0.920
Leu19- ¹³ Cδ2 (ppm)	24.147	24.261	24.085	24.149
Leu19- ¹ Hδ2 (ppm)	0.636	0.644	0.648	0.647
Thr23- ¹³ Cδ (ppm)	21.978	21.999	21.99	21.996
Thr23- ¹³ Hδ2 (ppm)	1.290	1.290	1.290	1.290
Leu28- ¹³ Cγ (ppm)	27.448	27.51	27.455	27.465
Leu28- ¹ Hγ (ppm)	2.039	2.046	2.030	2.032
Leu34- ¹³ Cγ (ppm)	27.081	27.116	27.058	27.085
Leu34- ¹ Hγ (ppm)	1.611	1.604	1.626	1.622
Glu47- ¹³ Cβ (ppm)	29.649	29.684	29.69	29.682
Glu47- ¹ Hβ (ppm)	2.082	2.085	2.082	2.081
Lys49- ¹³ Cγ (ppm)	24.588	24.644	24.654	24.629
Lys49- ¹ Hγ (ppm)	1.226	1.235	1.218	1.233
Leu74- ¹³ Cδ2 (ppm)	22.945	22.946	22.972	22.980
Leu74- ¹ Hδ2 (ppm)	0.552	0.556	0.557	0.564
Lys84- ¹³ Cδ (ppm)	29.163	29.232	29.274	29.264
Lys84- ¹ Hδ (ppm)	1.703	1.702	1.690	1.692
Lys91- ¹³ Cδ (ppm)	24.606	24.65	24.646	24.556
Lys91- ¹ Hδ (ppm)	1.143	1.146	1.160	1.156
Thr92- ¹³ Cδ (ppm)	21.940	21.939	21.94	21.940
Thr92- ¹³ Hδ2 (ppm)	1.235	1.235	1.235	1.235
Ile103- ¹³ Cγ2 (ppm)	17.656	17.585	17.593	17.583
Ile103- ¹ Hγ2 (ppm)	0.785	0.790	0.787	0.786
Leu104- ¹³ Cδ2 (ppm)	22.991	23.057	23.011	23.074
Leu104- ¹ Hδ2 (ppm)	0.361	0.363	0.359	0.361
Leu107- ¹³ Cδ2 (ppm)	23.342	23.342	23.461	23.433
Leu107- ¹ Hδ2 (ppm)	0.637	0.642	0.643	0.642

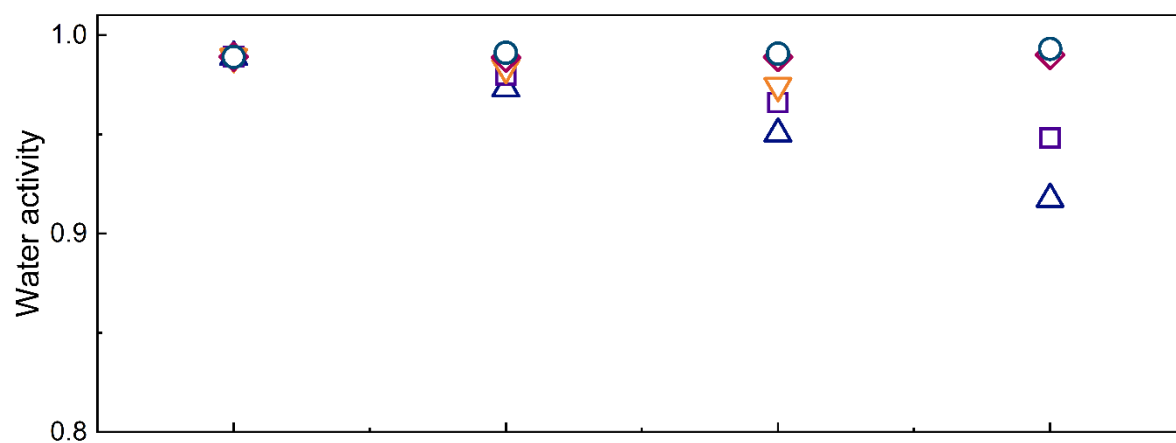


Fig. S11 The water activity of the stability samples containing 0, 10, 20, and 30% v/v fructose (Δ), sucrose (□), and melezitose (▽), 1.1, 5.5, and 10.9 mM DDM (◇), and 0.1, 0.4, and 0.8 mM polysorbate 80 (○) measured at 20°C. All samples contained 9 mg/mL GA-Z in PBS buffer (25 mM sodium phosphate, 125 mM NaCl, pH 7.0)