

## Supplementary Information

### Trehalose hydrogels for stabilization of enzymes to heat

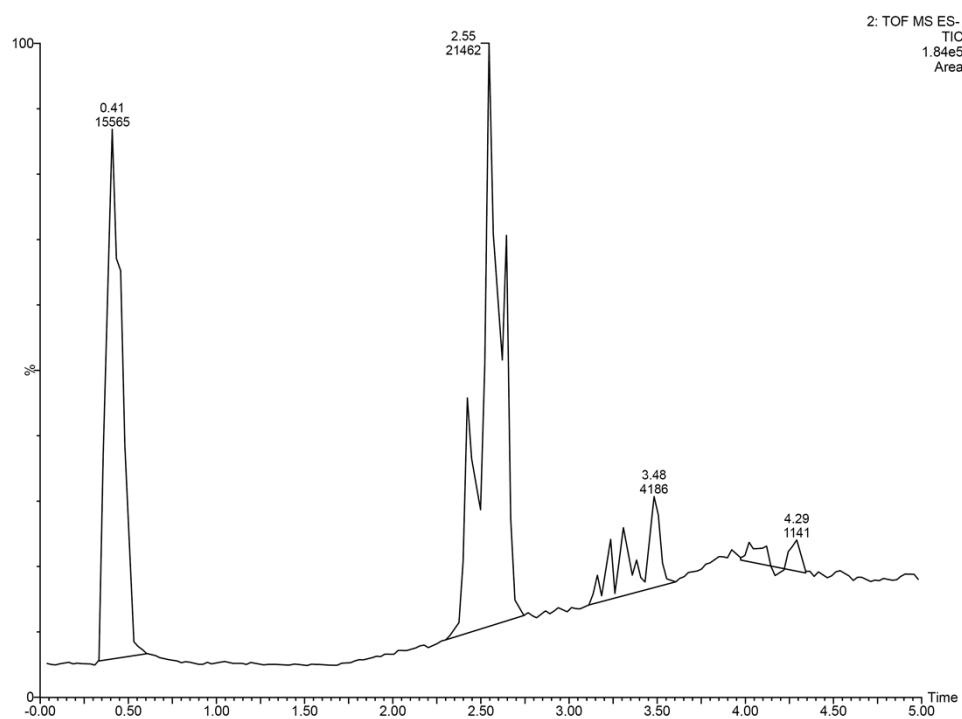
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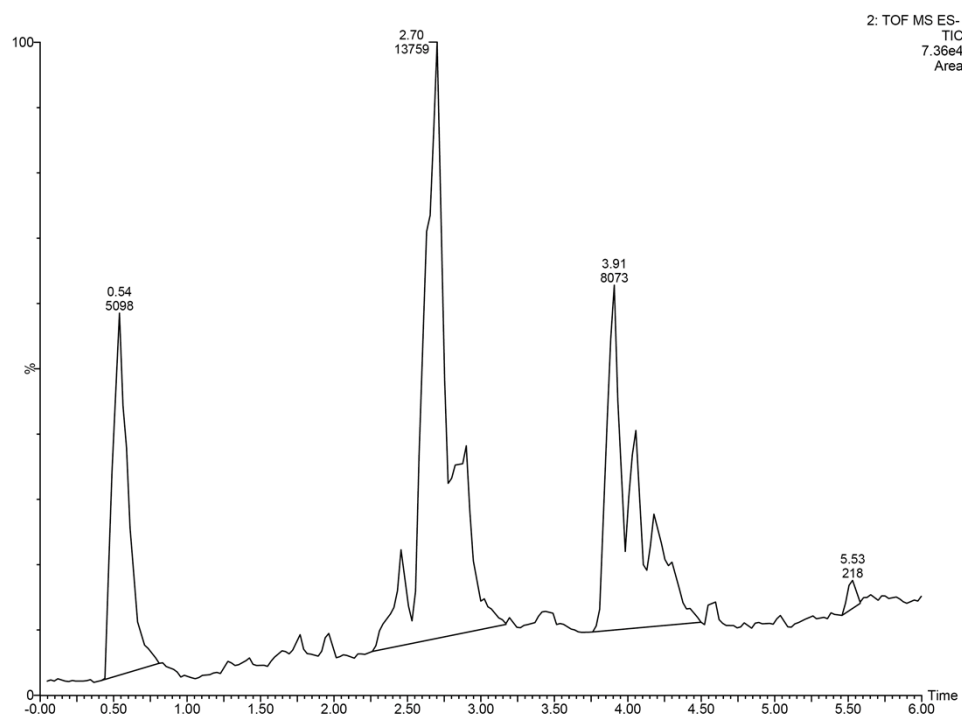
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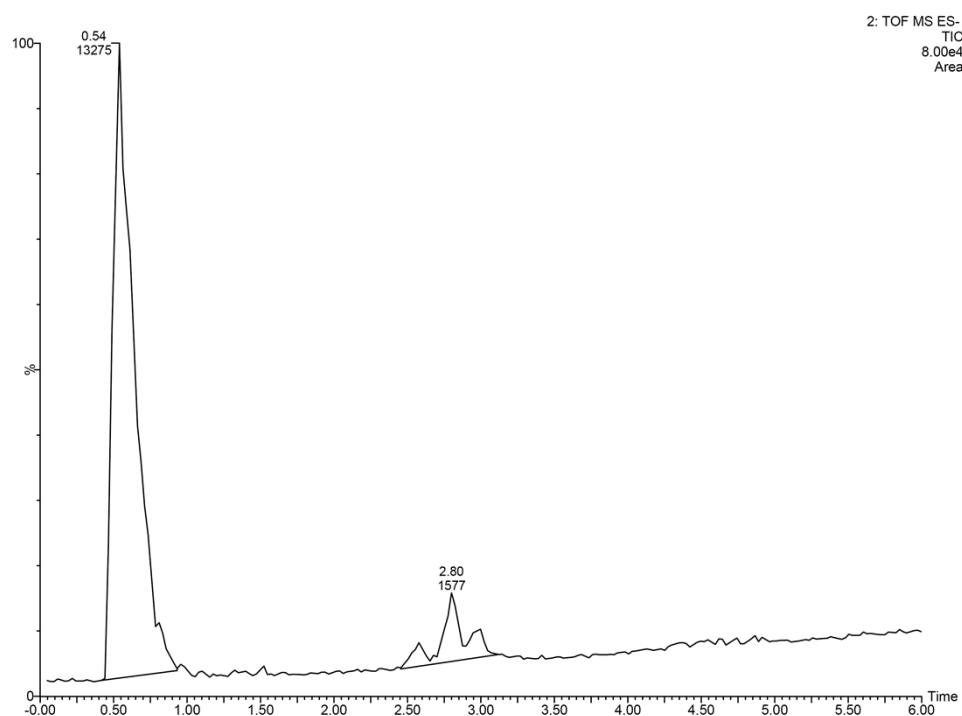
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**Figure S1.** LC-MS chromatogram of crude styrenyl ether trehalose mixture after precipitation in DCM.



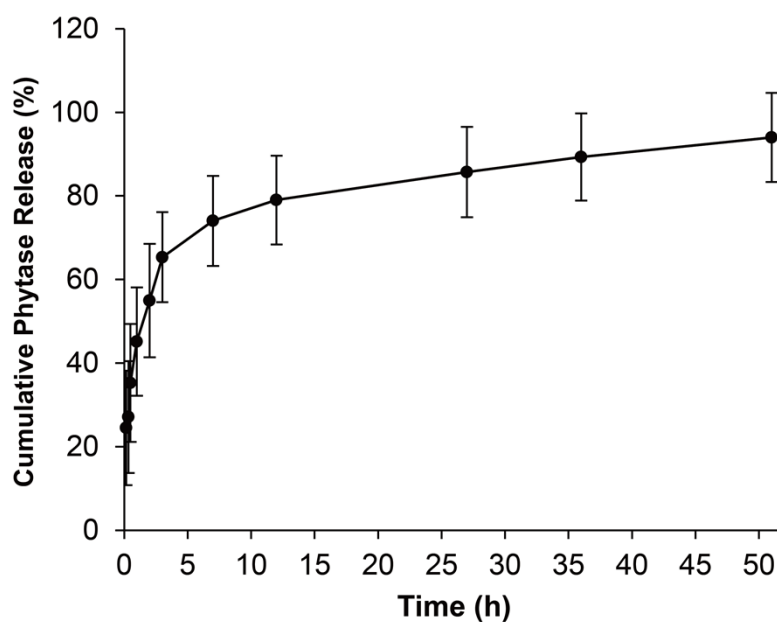
**Figure S2.** LC-MS chromatogram of the DCM wash of the crude styrenyl ether trehalose mixture.



**Figure S3.** LC-MS chromatogram of the trehalose hydrogel reaction mixture after 1 d.

### **Release of phytase from hydrogel that had not been lyophilized.**

FITC-labeled phytase (30 mg/mL) in 0.1 M sodium acetate buffer (pH 5.0) was added to 0.5 mg of trehalose hydrogel to fully hydrate the gel (25  $\mu$ L water per 1 mg of hydrogel). The mixture was incubated at room temperature for 12 h, and then 200  $\mu$ L buffer was added to initiate the passive diffusion of the phytase from the hydrogel. Half of the solution was removed at various time points and fresh buffer was added. The concentrations of the time point aliquots were calculated from the fluorescence measured on a spectrofluorometer using a FITC-labeled phytase calibration curve.



**Figure S4.** Release profile of FITC-labeled phytase from trehalose hydrogel prior to lyophilization (n = 6).

**Table S1.** Theoretical and observed masses of  $[M+HCOO]^-$  ion of trehalose and its derivatives from LC-MS chromatogram in Figure S2

	Retention time (min)	Theoretical mass (m/z)	Observed mass (m/z)	$\Delta$ m/z (ppm)
Trehalose	0.6	387.1139	387.1143	-1.1
Mono-substituted	2.5	503.1765	503.1762	0.5
	2.8	503.1765	503.1720	8.9
	2.9	503.1765	503.1765	-0.1
Di-substituted	4.4	619.2391	619.2369	3.5
Tri-substituted	5.5	735.3017	735.3012	0.6