Supplementary Information

Table S1. Inlet temperatures (T_{in}) required to achieve the outlet temperature (T_{out}) of the Box-Behnken DoE applied in this work. All solutions had a solids content of 0.1%. The atomising gas flow rate and drying gas flow rate was kept constant at 1374 L/h and 35 m^3 /h respectively.

DoE level	**T_{in} (°C)**	**T_{out} (°C)**	**Feed Flow rate (mL/min)**
--	95	70	1.50
-+	127	70	4.00
+-	136	100	1.50
++	150	100	4.00
Centre	128	85	2.75

Table S2. Particle size distributions (x_{50} and span, in µm) of spray dried powders of ALDH*Tt*-native and ALDH*Tt*-508 measured using scanning electron microscopy (SEM). Duplicate samples were produced, and 200 particles were measured in triplicate.

DoE level	ALDH*Tt*-native		ALDH*Tt*-508	
	x_{50} (µm)	Span (µm)	x_{50} (µm)	Span (µm)
--	5.45 ± 0.41	1.05 ± 0.04	5.55 ± 0.18	1.05 ± 0.10
- +	5.91 ± 0.09	0.97 ± 0.07	5.98 ± 0.27	1.00 ± 0.05
+ -	7.84 ± 0.41	0.90 ± 0.01	7.54 ± 0.91	0.87 ± 0.18
+ +	7.37 ± 0.69	0.94 ± 0.07	7.93 ± 1.49	1.04 ± 0.13
Centre	7.49 ± 0.52	0.87 ± 0.15	7.19 ± 0.26	1.02 ± 0.29

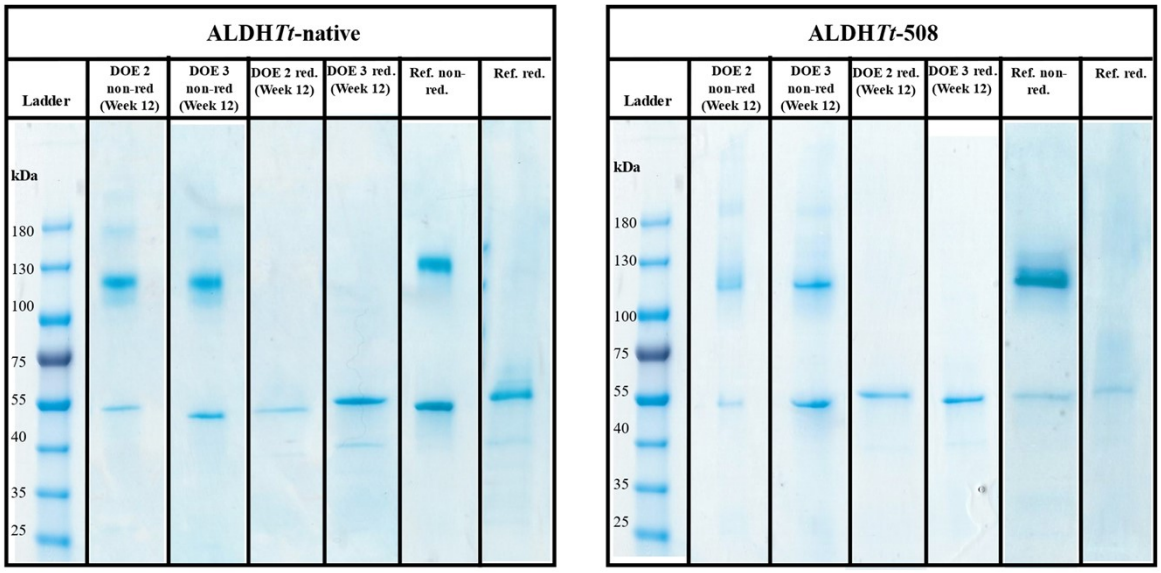

Fig. S1. SDS-PAGE (12%) of reduced and non-reduced ALDH*Tt*-native and ALDH*Tt*-508 after 12 weeks of storage. Untreated ALDH*Tt* protein was used as a reference.

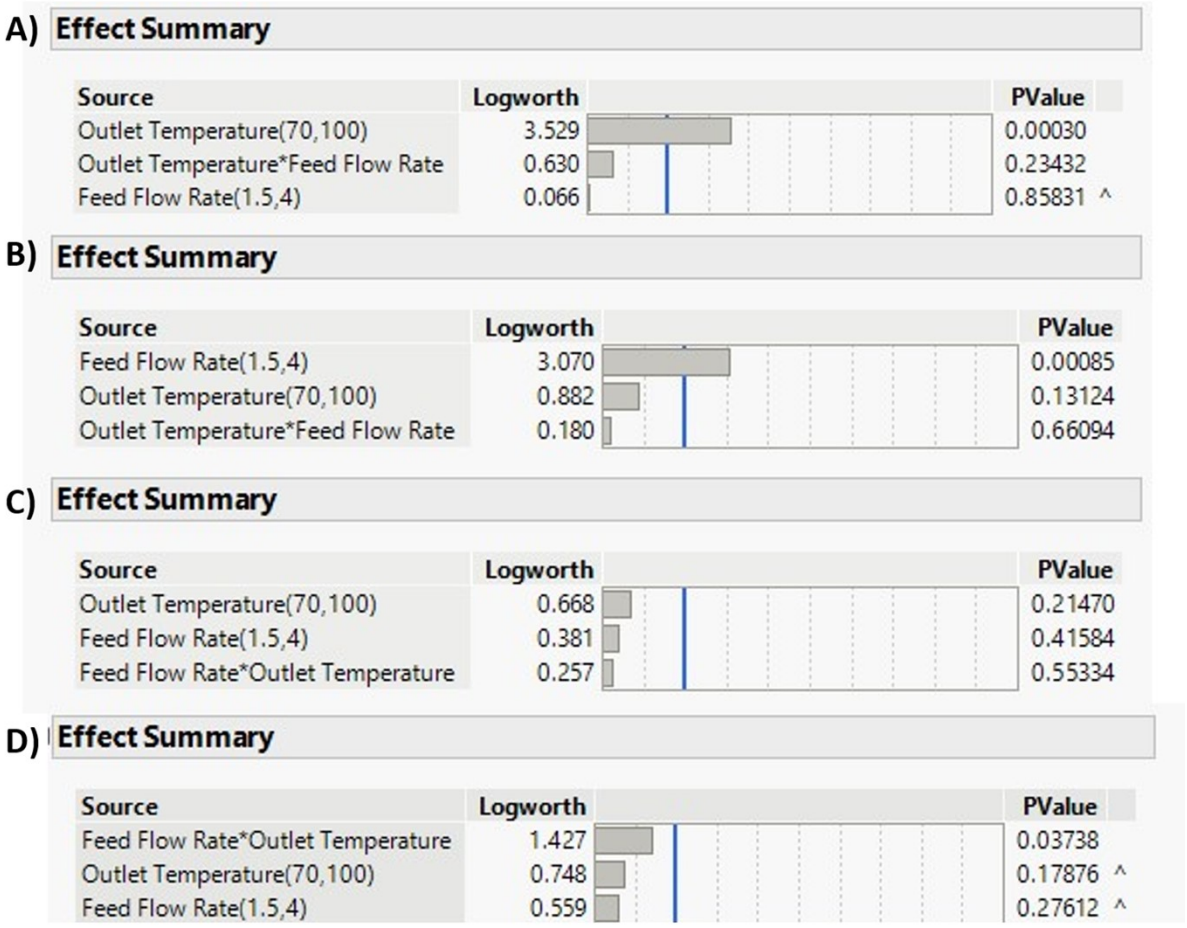

Fig. S2. Summary of the effects of input factors of outlet temperature (T_{out}) and feed flow rate (F_{flow}) on the outputs of A) particle size, B) moisture content, C) residual enzymatic activity and D) native structure retention.

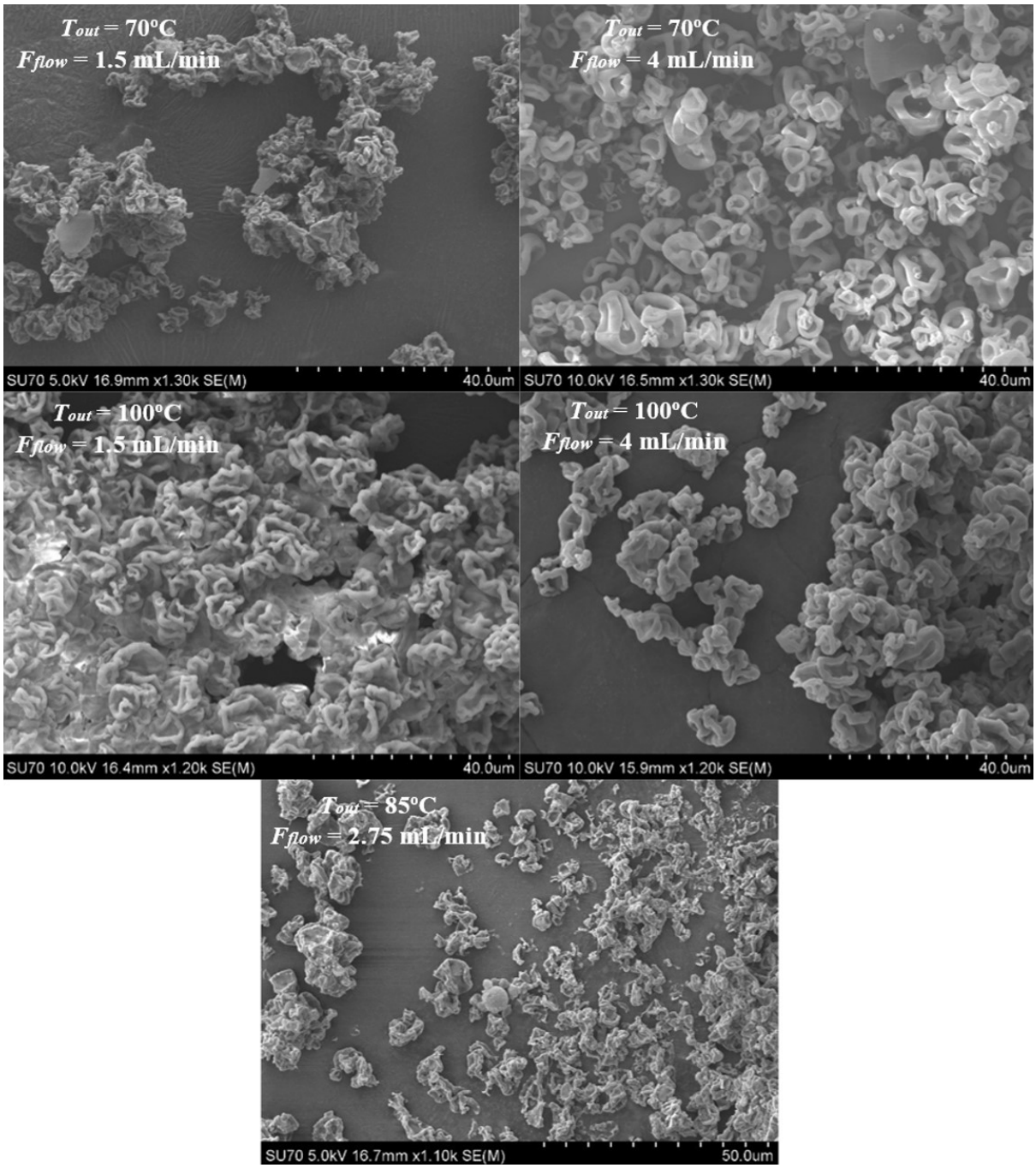

Fig. S3. Scanning electron microscopy (SEM) images of spray dried ALDH*Tt*-native under the process conditions of the Box-Behnken DoE.

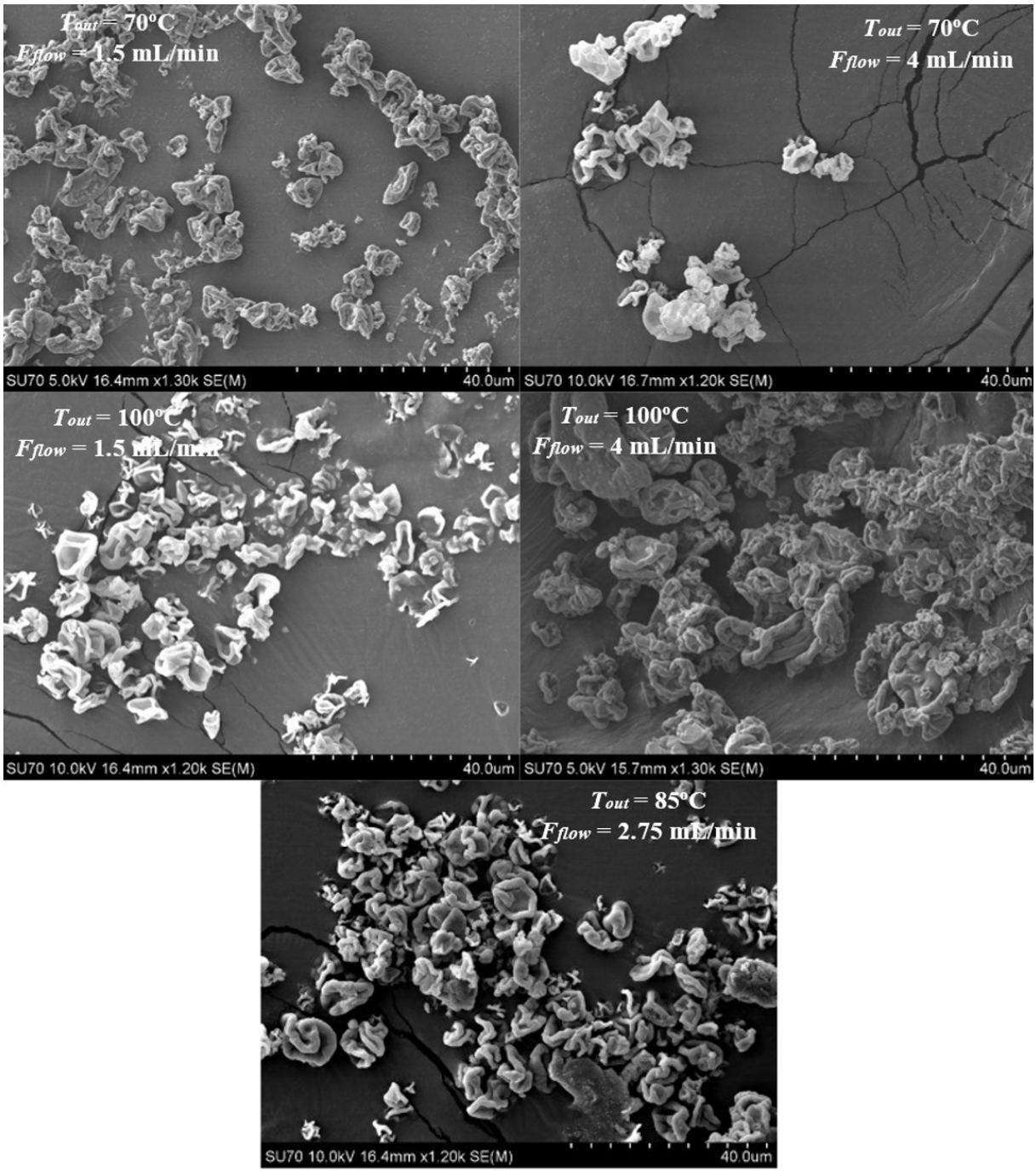

Fig. S4. Scanning Electron Microscopy (SEM) images of spray dried ALDH*Tt*-508 under process conditions of the Box-Behnken DoE. .

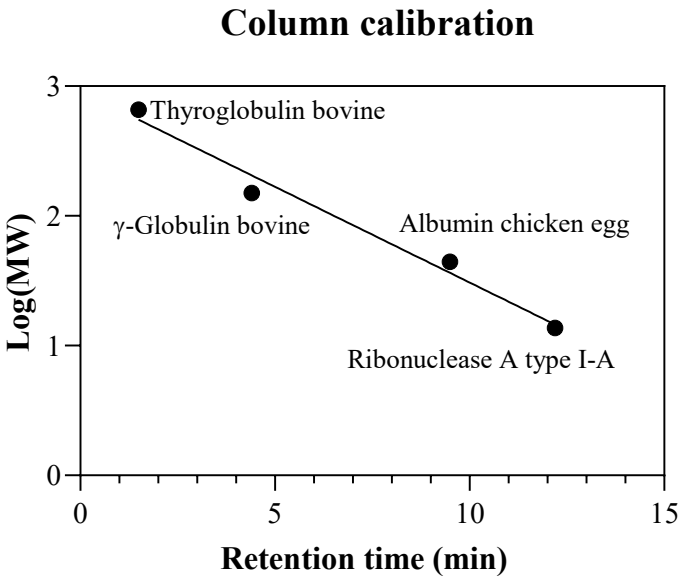

Figure S5. Calibration curve used for the molecular weight determination of ALDH*Tt* and its oligomeric states using Size Exclusion High Performance Liquid Chromatography (SE-HPLC).

Table S3. Mean and standard deviation values of turbidity of untreated and spray dried reconstituted solutions of ALDH*Tt*-native and ALDH*Tt*-508 values are reported in NTU (Nephelometric Turbidity Units).

Protein		**DoE Level**				
	Untreated	--	-+	+-	++	Centre
ALDH*Tt*-native	1.34 ± 0.17	4.48 ± 0.05	1.80 ± 0.08	1.40 ± 0.03	57.38 ± 5.19	1.27 ± 0.02
ALDH*Tt*-508	1.65 ± 0.03	9.85 ± 0.78	2.38 ± 0.12	8.15 ± 0.12	1.22 ± 1.12	1.98 ± 0.13

Table S4. Circular Dichroism (CD) spectrum secondary structure quantification using BestSel (https://bestsel.elte.hu) in the wavelength range 190-250 nm. The RMSD indicates the root mean standard deviation, and NRMSD indicates the normalised root mean standard deviation.

ALDH*Tt*-native	**α-helix (of which distorted)**	**β-sheet (anti-parallel)**	**β-sheet (parallel)**	**Turns**	**Others**	**RMSD**	**NRMSD**
	(%)	(%)	(%)	(%)	(%)	(-)	(-)
Ref.	16.2 (1.1)	20.4	9.8	15.1	38.5	0.2318	0.04581
RM (%) = 12%	13.6 (0.0)	31.5	11.8	15.3	27.8	0.4993	0.05223
RM (%) = 12% (W12)	7.5 (4.1)	27.3	6.5	14.5	44.2	0.0651	0.03239
Ref.	16.2 (1.1)	20.4	9.8	15.1	38.5	0.2318	0.04581
RM (%) = 6%	19.1 (1.4)	22.4	11.5	15.5	31.5	0.2504	0.04583
RM (%) = 6% (W12)	16.5 (4.1)	41.0	4.8	16.4	21.2	0.3705	0.02802
Thermally treated	8.9 (8.9)	29.4	0.0	13.6	48.1	0.3219	0.04534
ALDH*Tt*-508							
Ref.	22.9 (0.0)	36.4	8.2	16.0	16.6	0.7297	0.07358
RM (%) = 12%	19.6 (15.7)	26.8	1.5	14.1	37.9	0.4370	0.03910
RM (%) = 12% (W12)	24.8 (17.2)	28.2	0.0	12.3	34.6	0.4928	0.04100
Ref.	12.6 (0.0)	24.8	9.3	16.6	36.6	0.4497	0.06931
RM (%) = 6%	6.0 (2.5)	36.4	0.6	14.4	42.6	0.0290	0.02423
RM (%) = 6% (W12)	26.2 (20.9)	28.0	0.0	13.1	32.6	0.7098	0.04842
Thermally treated	0.0 (0.0)	35.8	5.7	17.7	40.9	0.5269	0.08248