






## Supplementary sheet

**Table S1.** DOE: Full factorial screening with 24 independent runs

	1	2	3	4	5	6	7	8
	Exp No	Exp Name	Run Order	Incl/Excl	Lyoprotectant	Lyoprot	Annealing	Bacterial concentration
1	1	N1	6	Incl	✓	Sucrose	✓	10 Yes
2	2	N2	15	Incl	✓	Trehalose	✓	10 Yes
3	3	N3	4	Incl	✓	Sucrose	✓	20 Yes
4	4	N4	8	Incl	✓	Trehalose	✓	20 Yes
5	5	N5	12	Incl	✓	Sucrose	✓	10 No
6	6	N6	3	Incl	✓	Trehalose	✓	10 No
7	7	N7	19	Incl	✓	Sucrose	✓	20 No
8	8	N8	9	Incl	✓	Trehalose	✓	20 No
9	9	N9	7	Incl	✓	Sucrose	✓	10 Yes
10	10	N10	18	Incl	✓	Trehalose	✓	10 Yes
11	11	N11	2	Incl	✓	Sucrose	✓	20 Yes
12	12	N12	5	Incl	✓	Trehalose	✓	20 Yes
13	13	N13	13	Incl	✓	Sucrose	✓	10 No
14	14	N14	16	Incl	✓	Trehalose	✓	10 No
15	15	N15	1	Incl	✓	Sucrose	✓	20 No
16	16	N16	10	Incl	✓	Trehalose	✓	20 No
17	17	N17	17	Incl	✓	Sucrose	✓	15 Yes
18	18	N18	11	Incl	✓	Trehalose	✓	15 Yes
19	19	N19	14	Incl	✓	Sucrose	✓	15 No
20	20	N20	20	Incl	✓	Trehalose	✓	15 No
21	21	N21	21	Incl	✓	Sucrose	✓	15 Yes
22	22	N22	22	Incl	✓	Trehalose	✓	15 Yes
23	23	N23	23	Incl	✓	Sucrose	✓	15 No
24	24	N24	24	Incl	✓	Trehalose	✓	15 No

**Table S2.** Description of the cake appearance and their corresponding scoring numbers

Score no.	Description	Image
1	Intact and homogenous cake	
2	Intact, heterogenous cake structure and color change of the cake (yellow to dark yellow or orange)	
3	Shrinkage of the cake around the edges and crust appearance of bottom part of the cake but no change in height of the cake	
4	Partial collapse, decrease in height of the cake < 60%	
5	Collapsed cake (decrease in height of >60%)	

**Table S3.** Tukey post-hoc tests: effects and interactions of the factors on water content of freeze-dried R2LC

**Post Hoc Tests- Water content**

**Post Hoc Comparisons - Lyoprotectant**

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose	Trehalose	0.597	0.157	3.807	0.004 **

\*\* p < .01

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration, Bacterial concentration

**Post Hoc Comparisons - Annealing**

		Mean Difference	SE	t	p <sub>tukey</sub>
No	Yes	-0.385	0.157	-2.457	0.036 *

\* p < .05

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration, Bacterial concentration

**Post Hoc Comparisons - Lyoprotectant concentration**

		Mean Difference	SE	t	p <sub>tukey</sub>
10	15	-0.220	0.192	-1.146	0.512
	20	-1.464	0.192	-7.626	< .001 ***
15	20	-1.244	0.192	-6.480	< .001 ***

\*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial concentration

**Post Hoc Comparisons - Bacterial concentration**

		Mean Difference	SE	t	p <sub>tukey</sub>
E10	E9	-0.825	0.157	-5.264	< .001 ***

\*\*\* p < .001

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration

**Post Hoc Comparisons - Lyoprotectant \* Annealing**

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose No	Trehalose No	0.235	0.222	1.060	0.720
	Sucrose Yes	-0.747	0.222	-3.369	0.034 *
	Trehalose Yes	0.212	0.222	0.955	0.777
Trehalose No	Sucrose Yes	-0.982	0.222	-4.429	0.007 **
	Trehalose Yes	-0.023	0.222	-0.105	1.000

### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose Yes	Trehalose Yes	0.958	0.222	4.324	0.009 **

\* p < .05, \*\* p < .01

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose 10	Trehalose 10	-0.218	0.271	-0.801	0.960
	Sucrose 15	-0.605	0.271	-2.229	0.311
	Trehalose 15	-0.053	0.271	-0.193	1.000
	Sucrose 20	-2.300	0.271	-8.473	< .001 ***
	Trehalose 20	-0.845	0.271	-3.113	0.094
Trehalose 10	Sucrose 15	-0.387	0.271	-1.428	0.712
	Trehalose 15	0.165	0.271	0.608	0.988
	Sucrose 20	-2.082	0.271	-7.672	< .001 ***
	Trehalose 20	-0.627	0.271	-2.312	0.280
Sucrose 15	Trehalose 15	0.552	0.271	2.035	0.393
	Sucrose 20	-1.695	0.271	-6.244	0.001 **
	Trehalose 20	-0.240	0.271	-0.884	0.941
Trehalose 15	Sucrose 20	-2.248	0.271	-8.280	< .001 ***
	Trehalose 20	-0.792	0.271	-2.920	0.123
Sucrose 20	Trehalose 20	1.455	0.271	5.360	0.004 **

\* p < .05, \*\* p < .01, \*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 6

*Note.* Results are averaged over the levels of: Annealing, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose E10	Trehalose E10	0.328	0.222	1.481	0.486
	Sucrose E9	-1.093	0.222	-4.933	0.004 **
	Trehalose E9	-0.228	0.222	-1.030	0.737
Trehalose E10	Sucrose E9	-1.422	0.222	-6.414	< .001 ***
	Trehalose E9	-0.557	0.222	-2.512	0.124
Sucrose E9	Trehalose E9	0.865	0.222	3.903	0.016 *

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration

*Note.* P-value adjusted for comparing a family of 4

\* p < .05, \*\* p < .01, \*\*\* p < .001

# Post Hoc Comparisons - Lyoprotectant \* Annealing \* Lyoprotectant concentration

		Mean Difference	SE	t	p <sub>Tukey</sub>
Sucrose No 10	Trehalose No 10	-0.215	0.163	-1.323	0.912
	Sucrose Yes 10	-0.210	0.163	-1.292	0.920
	Trehalose Yes 10	-0.430	0.163	-2.646	0.521
	Sucrose No 15	-0.575	0.163	-3.538	0.347
	Trehalose No 15	-0.185	0.163	-1.138	0.953
	Sucrose Yes 15	-0.845	0.163	-5.199	0.184
	Trehalose Yes 15	-0.130	0.163	-0.800	0.993
	Sucrose No 20	-1.525	0.163	-9.384	0.061
	Trehalose No 20	-0.995	0.163	-6.122	0.137
	Sucrose Yes 20	-3.285	0.163	-20.213	0.014 *
	Trehalose Yes 20	-0.905	0.163	-5.569	0.162
Trehalose No 10	Sucrose Yes 10	0.005	0.163	0.031	1.000
	Trehalose Yes 10	-0.215	0.163	-1.323	0.912
	Sucrose No 15	-0.360	0.163	-2.215	0.639
	Trehalose No 15	0.030	0.163	0.185	1.000
	Sucrose Yes 15	-0.630	0.163	-3.876	0.301
	Trehalose Yes 15	0.085	0.163	0.523	1.000
	Sucrose No 20	-1.310	0.163	-8.061	0.082
	Trehalose No 20	-0.780	0.163	-4.799	0.211
	Sucrose Yes 20	-3.070	0.163	-18.890	0.015 *
	Trehalose Yes 20	-0.690	0.163	-4.246	0.260
Sucrose Yes 10	Trehalose Yes 10	-0.220	0.163	-1.354	0.904
	Sucrose No 15	-0.365	0.163	-2.246	0.630
	Trehalose No 15	0.025	0.163	0.154	1.000
	Sucrose Yes 15	-0.635	0.163	-3.907	0.297
	Trehalose Yes 15	0.080	0.163	0.492	1.000
	Sucrose No 20	-1.315	0.163	-8.091	0.081
	Trehalose No 20	-0.785	0.163	-4.830	0.209
	Sucrose Yes 20	-3.075	0.163	-18.921	0.015 *
	Trehalose Yes 20	-0.695	0.163	-4.276	0.257
Trehalose Yes 10	Sucrose No 15	-0.145	0.163	-0.892	0.987
	Trehalose No 15	0.245	0.163	1.508	0.861
	Sucrose Yes 15	-0.415	0.163	-2.554	0.545
	Trehalose Yes 15	0.300	0.163	1.846	0.754
	Sucrose No 20	-1.095	0.163	-6.738	0.115
	Trehalose No 20	-0.565	0.163	-3.477	0.357
	Sucrose Yes 20	-2.855	0.163	-17.567	0.017 *
	Trehalose Yes 20	-0.475	0.163	-2.923	0.458
Sucrose No 15	Trehalose No 15	0.390	0.163	2.400	0.586
	Sucrose Yes 15	-0.270	0.163	-1.661	0.813
	Trehalose Yes 15	0.445	0.163	2.738	0.499
	Sucrose No 20	-0.950	0.163	-5.845	0.149
	Trehalose No 20	-0.420	0.163	-2.584	0.537

**Post Hoc Comparisons - Lyoprotectant \* Annealing \* Lyoprotectant concentration**

		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>Tukey</sub></b>
Trehalose No 15	Sucrose Yes 20	-2.710	0.163	-16.675	0.019 *
	Trehalose Yes 20	-0.330	0.163	-2.031	0.695
	Sucrose Yes 15	-0.660	0.163	-4.061	0.279
	Trehalose Yes 15	0.055	0.163	0.338	1.000
	Sucrose No 20	-1.340	0.163	-8.245	0.079
	Trehalose No 20	-0.810	0.163	-4.984	0.198
Sucrose Yes 15	Sucrose Yes 20	-3.100	0.163	-19.075	0.015 *
	Trehalose Yes 20	-0.720	0.163	-4.430	0.242
	Trehalose Yes 15	0.715	0.163	4.399	0.245
	Sucrose No 20	-0.680	0.163	-4.184	0.266
	Trehalose No 20	-0.150	0.163	-0.923	0.984
Trehalose Yes 15	Sucrose Yes 20	-2.440	0.163	-15.014	0.024 *
	Trehalose Yes 20	-0.060	0.163	-0.369	1.000
	Sucrose No 20	-1.395	0.163	-8.584	0.073
	Trehalose No 20	-0.865	0.163	-5.322	0.176
Sucrose No 20	Sucrose Yes 20	-3.155	0.163	-19.413	0.015 *
	Trehalose Yes 20	-0.775	0.163	-4.769	0.213
	Trehalose No 20	0.530	0.163	3.261	0.392
	Sucrose Yes 20	-1.760	0.163	-10.829	0.047 *
Trehalose No 20	Trehalose Yes 20	0.620	0.163	3.815	0.309
	Sucrose Yes 20	-2.290	0.163	-14.091	0.028 *
	Trehalose Yes 20	0.090	0.163	0.554	1.000
Sucrose Yes 20	Trehalose Yes 20	2.380	0.163	14.644	0.025 *

\* p < .05

*Note.* P-value adjusted for comparing a family of 12

*Note.* Results are averaged over the levels of: Bacterial concentration

**Table S4.** Tukey post-hoc tests: effects and interactions of the factors on Tg of freeze-dried R2LC

## Post Hoc Tests- Glass transition temperature

### Standard

#### Post Hoc Comparisons - Lyoprotectant

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose	Trehalose	-33.803	2.122	-15.930	< .001 ***

\*\*\* p < .001

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Annealing

		Mean Difference	SE	t	p <sub>tukey</sub>
No	Yes	1.450	2.133	0.680	0.500

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Lyoprotectant concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
10	15	1.160	2.542	0.456	0.892
	20	4.438	2.681	1.655	0.235
15	20	3.278	2.602	1.260	0.426

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial concentration

#### Post Hoc Comparisons - Bacterial concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
E10	E9	5.743	2.125	2.703	0.010 **

\*\* p < .01

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration

#### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose No	Trehalose No	-34.415	3.157	-10.900	< .001 ***
	Sucrose Yes	0.838	3.015	0.278	0.992
	Trehalose Yes	-32.353	3.002	-10.777	< .001 ***
Trehalose No	Sucrose Yes	35.253	3.015	11.693	< .001 ***
	Trehalose Yes	2.062	3.002	0.687	0.902

### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose Yes	Trehalose Yes	-33.191	2.836	-11.705	< .001 ***

\*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose 10	Trehalose 10	-30.949	3.714	-8.333	< .001 ***
	Sucrose 15	2.214	3.602	0.615	0.989
	Trehalose 15	-30.844	3.711	-8.312	< .001 ***
	Sucrose 20	7.664	3.867	1.982	0.370
Trehalose 10	Trehalose 20	-29.738	3.867	-7.690	< .001 ***
	Sucrose 15	33.163	3.456	9.597	< .001 ***
	Trehalose 15	0.105	3.524	0.030	1.000
	Sucrose 20	38.613	3.714	10.396	< .001 ***
Sucrose 15	Trehalose 20	1.212	3.714	0.326	0.999
	Trehalose 15	-33.059	3.385	-9.767	< .001 ***
	Sucrose 20	5.449	3.602	1.513	0.658
	Trehalose 20	-31.952	3.602	-8.871	< .001 ***
Trehalose 15	Sucrose 20	38.508	3.711	10.377	< .001 ***
	Trehalose 20	1.107	3.711	0.298	1.000
Sucrose 20	Trehalose 20	-37.401	3.867	-9.672	< .001 ***

\*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 6

*Note.* Results are averaged over the levels of: Annealing, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose E10	Trehalose E10	-31.530	2.866	-11.001	< .001 ***
	Sucrose E9	8.016	2.979	2.691	0.048 *
	Trehalose E9	-28.060	3.050	-9.200	< .001 ***
Trehalose E10	Sucrose E9	39.546	2.955	13.382	< .001 ***
	Trehalose E9	3.470	3.002	1.156	0.658
Sucrose E9	Trehalose E9	-36.076	3.106	-11.614	< .001 ***

\* p < .05, \*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration



# **Post Hoc Comparisons - Lyoprotectant \* Annealing \* Bacterial concentration**

		Mean Difference	SE	t	p <sub>Tukey</sub>
Sucrose No E10	Trehalose No E10	-34.455	4.337	-7.944	< .001 ***
	Sucrose Yes E10	6.399	4.102	1.560	0.770
	Trehalose Yes E10	-24.475	3.951	-6.195	< .001 ***
	Sucrose No E9	12.522	4.337	2.887	0.107
	Trehalose No E9	-21.853	4.337	-5.038	< .001 ***
	Sucrose Yes E9	8.743	4.211	2.076	0.450
	Trehalose Yes E9	-28.923	4.337	-6.668	< .001 ***
Trehalose No E10	Sucrose Yes E10	40.854	4.102	9.960	< .001 ***
	Trehalose Yes E10	9.980	3.951	2.526	0.219
	Sucrose No E9	46.977	4.337	10.831	< .001 ***
	Trehalose No E9	12.602	4.337	2.905	0.103
	Sucrose Yes E9	43.198	4.211	10.258	< .001 ***
	Trehalose Yes E9	5.532	4.337	1.275	0.902
Sucrose Yes E10	Trehalose Yes E10	-30.874	3.679	-8.393	< .001 ***
	Sucrose No E9	6.123	4.102	1.493	0.806
	Trehalose No E9	-28.252	4.102	-6.888	< .001 ***
	Sucrose Yes E9	2.344	3.953	0.593	0.999
	Trehalose Yes E9	-35.322	4.102	-8.612	< .001 ***
Trehalose Yes E10	Sucrose No E9	36.997	3.951	9.364	< .001 ***
	Trehalose No E9	2.622	3.951	0.664	0.997
	Sucrose Yes E9	33.217	3.818	8.700	< .001 ***
	Trehalose Yes E9	-4.448	3.951	-1.126	0.946
Sucrose No E9	Trehalose No E9	-34.375	4.337	-7.925	< .001 ***
	Sucrose Yes E9	-3.779	4.211	-0.897	0.984
	Trehalose Yes E9	-41.445	4.337	-9.555	< .001 ***
Trehalose No E9	Sucrose Yes E9	30.596	4.211	7.265	< .001 ***
	Trehalose Yes E9	-7.070	4.337	-1.630	0.729
Sucrose Yes E9	Trehalose Yes E9	-37.666	4.211	-8.944	< .001 ***

\*\* p < .01, \*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 8

*Note.* Results are averaged over the levels of: Lyoprotectant concentration

**Table S5.** Tukey post-hoc tests: effects and interactions of the factors on aggregation of freeze-dried R2LC

## Post Hoc Tests-Aggregation

### Standard

#### Post Hoc Comparisons - Lyoprotectant

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose	Trehalose	-0.139	0.060	-2.329	0.022 *

\*  $p < .05$

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Annealing

		Mean Difference	SE	t	P <sub>tukey</sub>
No	Yes	0.089	0.060	1.500	0.137

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
10	15	-0.142	0.073	-1.958	0.129
	20	0.118	0.073	1.607	0.248
15	20	0.260	0.073	3.576	0.002 **

\*\*  $p < .01$

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial concentration

#### Post Hoc Comparisons - Bacterial concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
E10	E9	1.488	0.060	25.012	< .001 ***

\*\*\*  $p < .001$

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration

#### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose No	Trehalose No	-0.355	0.084	-4.232	< .001 ***
	Sucrose Yes	-0.127	0.084	-1.512	0.435
	Trehalose Yes	-0.049	0.084	-0.589	0.935
Trehalose No	Sucrose Yes	0.228	0.085	2.695	0.042 *

### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	P <sub>Tukey</sub>
Sucrose Yes	Trehalose Yes	0.305	0.085	3.610	0.003 **
	Trehalose Yes	0.077	0.085	0.914	0.797

\*  $p < .05$ , \*\*  $p < .01$ , \*\*\*  $p < .001$

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>Tukey</sub>
Sucrose 10	Trehalose 10	-0.166	0.104	-1.608	0.596
	Sucrose 15	-0.224	0.102	-2.194	0.252
	Trehalose 15	-0.227	0.104	-2.194	0.252
	Sucrose 20	0.158	0.104	1.521	0.652
	Trehalose 20	-0.089	0.104	-0.857	0.956
Trehalose 10	Sucrose 15	-0.058	0.102	-0.563	0.993
	Trehalose 15	-0.061	0.104	-0.586	0.992
	Sucrose 20	0.324	0.104	3.129	0.028 *
	Trehalose 20	0.078	0.104	0.752	0.975
Sucrose 15	Trehalose 15	-0.003	0.102	-0.031	1.000
	Sucrose 20	0.382	0.102	3.736	0.005 **
	Trehalose 20	0.135	0.102	1.325	0.770
Trehalose 15	Sucrose 20	0.385	0.104	3.715	0.005 **
	Trehalose 20	0.138	0.104	1.338	0.763
Sucrose 20	Trehalose 20	-0.246	0.104	-2.378	0.176

\*  $p < .05$ , \*\*  $p < .01$

*Note.* P-value adjusted for comparing a family of 6

*Note.* Results are averaged over the levels of: Annealing, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	P <sub>Tukey</sub>
Sucrose E10	Trehalose E10	0.028	0.085	0.333	0.987
	Sucrose E9	1.655	0.084	19.757	< .001 ***
	Trehalose E9	1.350	0.085	15.966	< .001 ***
Trehalose E10	Sucrose E9	1.627	0.084	19.421	< .001 ***
	Trehalose E9	1.322	0.085	15.634	< .001 ***
Sucrose E9	Trehalose E9	-0.305	0.084	-3.645	0.003 **

\*\*  $p < .01$ , \*\*\*  $p < .001$

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration

**Table S6.** Porosity of freeze-dried R2LC and an ANOVA and Tukey post-hoc tests: effects and interactions of the factors on porosity of freeze-dried R2LC

S.No.	Lyoprotectant	Lyoprotectant concentration (%)	Bacterial concentration (CFU/mL)	Annealing	Porosity (%)
1	Trehalose	10	$10^9$	Yes	10.2
2	Sucrose	10	$10^9$	Yes	13.42
3	Trehalose	10	$10^9$	No	10.96
4	Sucrose	10	$10^9$	No	33.5
5	Trehalose	20	$10^9$	Yes	10.51
6	Sucrose	20	$10^9$	Yes	10.26
7	Trehalose	20	$10^9$	No	9.72
8	Sucrose	20	$10^9$	No	13.47
9	Trehalose	10	$10^{10}$	Yes	9.58
10	Sucrose	10	$10^{10}$	Yes	18.91
11	Trehalose	10	$10^{10}$	No	17.29
12	Sucrose	10	$10^{10}$	No	9.43
13	Trehalose	20	$10^{10}$	Yes	20.48
14	Sucrose	20	$10^{10}$	Yes	15.54
15	Trehalose	20	$10^{10}$	No	17.7
16	Sucrose	20	$10^{10}$	No	13.66
17	Trehalose	15	$10^9$	Yes	11.61
18	Sucrose	15	$10^9$	Yes	12.38
19	Trehalose	15	$10^9$	No	16.92
20	Sucrose	15	$10^9$	No	11.99
21	Trehalose	15	$10^{10}$	Yes	12.83
22	Sucrose	15	$10^{10}$	Yes	28.44
23	Trehalose	15	$10^{10}$	No	15.99
24	Sucrose	15	$10^{10}$	No	12.93

## ANOVA - Porosity

Cases	Sum of Squares	df	Mean Square	F	p	$\eta^2_p$
Lyoprotectant	64.092	1	64.092	1.740	0.318	0.465
Annealing	0.004	1	0.004	$1.159 \times 10^{-4}$	0.992	$5.792 \times 10^{-5}$
Lyoprotectant concentration	11.704	2	5.852	0.159	0.863	0.137
Bacterial concentration	32.294	1	32.294	0.877	0.448	0.305
Lyoprotectant * Annealing	12.528	1	12.528	0.340	0.619	0.145
Lyoprotectant * Lyoprotectant concentration	70.496	2	35.248	0.957	0.511	0.489
Annealing * Lyoprotectant concentration	80.154	2	40.077	1.088	0.479	0.521
Lyoprotectant * Annealing * Lyoprotectant concentration	65.277	2	32.639	0.886	0.530	0.470
Lyoprotectant * Bacterial concentration	35.381	1	35.381	0.961	0.430	0.324
Annealing * Bacterial concentration	59.787	1	59.787	1.623	0.331	0.448
Lyoprotectant concentration * Bacterial concentration	94.340	2	47.170	1.281	0.438	0.562
Lyoprotectant * Annealing * Bacterial concentration	115.194	1	115.194	3.128	0.219	0.610
Lyoprotectant * Lyoprotectant concentration * Bacterial concentration	65.116	2	32.558	0.884	0.531	0.469
Annealing * Lyoprotectant concentration * Bacterial concentration	18.765	2	9.382	0.255	0.797	0.203
Residuals	73.657	2	36.829			

Note. Type III Sum of Squares

## Post Hoc Tests-porosity

### Standard

#### Post Hoc Comparisons - Lyoprotectant

	Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose      Trehalose	3.268	2.478	1.319	0.318

Note. Results are averaged over the levels of: Annealing, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Annealing

	Mean Difference	SE	t	p <sub>tukey</sub>
No      Yes	0.027	2.478	0.011	0.992

**Post Hoc Comparisons - Annealing**

		Mean Difference	SE	t	p <sub>Tukey</sub>
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*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration, Bacterial concentration

**Post Hoc Comparisons - Lyoprotectant concentration**

		Mean Difference	SE	t	p <sub>Tukey</sub>
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10	15	0.025	3.034	0.008	1.000
	20	1.494	3.034	0.492	0.882
15	20	1.469	3.034	0.484	0.886

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial concentration

**Post Hoc Comparisons - Bacterial concentration**

		Mean Difference	SE	t	p <sub>Tukey</sub>
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E10	E9	2.320	2.478	0.936	0.448
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*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration

**Post Hoc Comparisons - Lyoprotectant \* Annealing**

		Mean Difference	SE	t	p <sub>Tukey</sub>
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Sucrose No	Trehalose No	1.823	3.504	0.520	0.947
	Sucrose Yes	-1.418	3.504	-0.405	0.973
	Trehalose Yes	3.295	3.504	0.940	0.794
Trehalose No	Sucrose Yes	-3.242	3.504	-0.925	0.800
	Trehalose Yes	1.472	3.504	0.420	0.970
Sucrose Yes	Trehalose Yes	4.713	3.504	1.345	0.623

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration, Bacterial concentration

**Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration**

		Mean Difference	SE	t	p <sub>Tukey</sub>
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Sucrose 10	Trehalose 10	6.807	4.291	1.586	0.670
	Sucrose 15	1.245	4.291	0.290	0.999
	Trehalose 15	5.612	4.291	1.308	0.775
	Sucrose 20	5.582	4.291	1.301	0.778
	Trehalose 20	4.212	4.291	0.982	0.893
Trehalose 10	Sucrose 15	-5.562	4.291	-1.296	0.780
	Trehalose 15	-1.195	4.291	-0.278	0.999
	Sucrose 20	-1.225	4.291	-0.285	0.999
	Trehalose 20	-2.595	4.291	-0.605	0.980
Sucrose 15	Trehalose 15	4.367	4.291	1.018	0.881

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>Tukey</sub>
Trehalose 15	Sucrose 20	4.337	4.291	1.011	0.883
	Trehalose 20	2.967	4.291	0.692	0.966
	Sucrose 20	-0.030	4.291	-0.007	1.000
	Trehalose 20	-1.400	4.291	-0.326	0.999
Sucrose 20	Trehalose 20	-1.370	4.291	-0.319	0.999

*Note.* P-value adjusted for comparing a family of 6

*Note.* Results are averaged over the levels of: Annealing, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Annealing \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>Tukey</sub>
Sucrose No 10	Trehalose No 10	7.340	6.069	1.209	0.939
	Sucrose Yes 10	5.300	6.069	0.873	0.988
	Trehalose Yes 10	11.575	6.069	1.907	0.734
	Sucrose No 15	9.005	6.069	1.484	0.868
	Trehalose No 15	7.280	6.069	1.200	0.941
	Sucrose Yes 15	-1.215	6.069	-0.200	1.000
	Trehalose Yes 15	9.245	6.069	1.523	0.856
	Sucrose No 20	7.900	6.069	1.302	0.917
	Trehalose No 20	7.755	6.069	1.278	0.923
	Sucrose Yes 20	8.565	6.069	1.411	0.889
	Trehalose Yes 20	5.970	6.069	0.984	0.977
Trehalose No 10	Sucrose Yes 10	-2.040	6.069	-0.336	1.000
	Trehalose Yes 10	4.235	6.069	0.698	0.997
	Sucrose No 15	1.665	6.069	0.274	1.000
	Trehalose No 15	-0.060	6.069	-0.010	1.000
	Sucrose Yes 15	-8.555	6.069	-1.410	0.889
	Trehalose Yes 15	1.905	6.069	0.314	1.000
	Sucrose No 20	0.560	6.069	0.092	1.000
	Trehalose No 20	0.415	6.069	0.068	1.000
	Sucrose Yes 20	1.225	6.069	0.202	1.000
	Trehalose Yes 20	-1.370	6.069	-0.226	1.000
Sucrose Yes 10	Trehalose Yes 10	6.275	6.069	1.034	0.970
	Sucrose No 15	3.705	6.069	0.611	0.999
	Trehalose No 15	1.980	6.069	0.326	1.000
	Sucrose Yes 15	-6.515	6.069	-1.074	0.964
	Trehalose Yes 15	3.945	6.069	0.650	0.998
	Sucrose No 20	2.600	6.069	0.428	1.000
	Trehalose No 20	2.455	6.069	0.405	1.000
	Sucrose Yes 20	3.265	6.069	0.538	1.000
Trehalose Yes 10	Trehalose Yes 20	0.670	6.069	0.110	1.000
	Sucrose No 15	-2.570	6.069	-0.423	1.000
	Trehalose No 15	-4.295	6.069	-0.708	0.997

### Post Hoc Comparisons - Lyoprotectant \* Annealing \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose No 15	Sucrose Yes 15	-12.790	6.069	-2.108	0.671
	Trehalose Yes 15	-2.330	6.069	-0.384	1.000
	Sucrose No 20	-3.675	6.069	-0.606	0.999
	Trehalose No 20	-3.820	6.069	-0.629	0.999
	Sucrose Yes 20	-3.010	6.069	-0.496	1.000
	Trehalose Yes 20	-5.605	6.069	-0.924	0.984
Trehalose No 15	Trehalose No 15	-1.725	6.069	-0.284	1.000
	Sucrose Yes 15	-10.220	6.069	-1.684	0.806
	Trehalose Yes 15	0.240	6.069	0.040	1.000
	Sucrose No 20	-1.105	6.069	-0.182	1.000
	Trehalose No 20	-1.250	6.069	-0.206	1.000
	Sucrose Yes 20	-0.440	6.069	-0.073	1.000
Sucrose Yes 15	Trehalose Yes 20	-3.035	6.069	-0.500	1.000
	Sucrose Yes 15	-8.495	6.069	-1.400	0.892
	Trehalose Yes 15	1.965	6.069	0.324	1.000
	Sucrose No 20	0.620	6.069	0.102	1.000
	Trehalose No 20	0.475	6.069	0.078	1.000
	Sucrose Yes 20	1.285	6.069	0.212	1.000
Trehalose Yes 15	Trehalose Yes 20	-1.310	6.069	-0.216	1.000
	Trehalose Yes 15	10.460	6.069	1.724	0.794
	Sucrose No 20	9.115	6.069	1.502	0.863
	Trehalose No 20	8.970	6.069	1.478	0.870
	Sucrose Yes 20	9.780	6.069	1.612	0.829
	Trehalose Yes 20	7.185	6.069	1.184	0.944
Sucrose No 20	Sucrose No 20	-1.345	6.069	-0.222	1.000
	Trehalose No 20	-1.490	6.069	-0.246	1.000
	Sucrose Yes 20	-0.680	6.069	-0.112	1.000
	Trehalose Yes 20	-3.275	6.069	-0.540	1.000
	Trehalose No 20	-0.145	6.069	-0.024	1.000
	Sucrose Yes 20	0.665	6.069	0.110	1.000
Trehalose No 20	Trehalose Yes 20	-1.930	6.069	-0.318	1.000
	Sucrose Yes 20	0.810	6.069	0.133	1.000
	Trehalose Yes 20	-1.785	6.069	-0.294	1.000
	Sucrose Yes 20	-2.595	6.069	-0.428	1.000
	Trehalose Yes 20				
	Trehalose Yes 20				

Note. P-value adjusted for comparing a family of 12

Note. Results are averaged over the levels of: Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose E10	Trehalose E10	0.840	3.504	0.240	0.994
	Sucrose E9	-0.108	3.504	-0.031	1.000
	Trehalose E9	5.588	3.504	1.595	0.529



### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	P <sub>Tukey</sub>
Trehalose E10	Sucrose E9	-0.948	3.504	-0.271	0.991
	Trehalose E9	4.748	3.504	1.355	0.619
Sucrose E9	Trehalose E9	5.697	3.504	1.626	0.518

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration

### Post Hoc Comparisons - Lyoprotectant \* Annealing \* Bacterial concentration

		Mean Difference	SE	t	P <sub>Tukey</sub>
Sucrose No E10	Trehalose No E10	-4.987	4.955	-1.006	0.935
	Sucrose Yes E10	-8.957	4.955	-1.808	0.673
	Trehalose Yes E10	-2.290	4.955	-0.462	0.999
	Sucrose No E9	-7.647	4.955	-1.543	0.765
	Trehalose No E9	0.987	4.955	0.199	1.000
	Sucrose Yes E9	-1.527	4.955	-0.308	1.000
	Trehalose Yes E9	1.233	4.955	0.249	1.000
Trehalose No E10	Sucrose Yes E10	-3.970	4.955	-0.801	0.975
	Trehalose Yes E10	2.697	4.955	0.544	0.996
	Sucrose No E9	-2.660	4.955	-0.537	0.997
	Trehalose No E9	5.973	4.955	1.206	0.880
	Sucrose Yes E9	3.460	4.955	0.698	0.987
	Trehalose Yes E9	6.220	4.955	1.255	0.864
Sucrose Yes E10	Trehalose Yes E10	6.667	4.955	1.345	0.834
	Sucrose No E9	1.310	4.955	0.264	1.000
	Trehalose No E9	9.943	4.955	2.007	0.607
	Sucrose Yes E9	7.430	4.955	1.499	0.781
	Trehalose Yes E9	10.190	4.955	2.056	0.592
Trehalose Yes E10	Sucrose No E9	-5.357	4.955	-1.081	0.916
	Trehalose No E9	3.277	4.955	0.661	0.990
	Sucrose Yes E9	0.763	4.955	0.154	1.000
	Trehalose Yes E9	3.523	4.955	0.711	0.985
Sucrose No E9	Trehalose No E9	8.633	4.955	1.742	0.695
	Sucrose Yes E9	6.120	4.955	1.235	0.871
	Trehalose Yes E9	8.880	4.955	1.792	0.678
Trehalose No E9	Sucrose Yes E9	-2.513	4.955	-0.507	0.998
	Trehalose Yes E9	0.247	4.955	0.050	1.000
Sucrose Yes E9	Trehalose Yes E9	2.760	4.955	0.557	0.996

*Note.* P-value adjusted for comparing a family of 8

*Note.* Results are averaged over the levels of: Lyoprotectant concentration

**Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration \* Bacterial concentration**

		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
Sucrose 10 E10	Trehalose 10 E10	0.735	6.069	0.121	1.000
	Sucrose 15 E10	-6.515	6.069	-1.074	0.964
	Trehalose 15 E10	-0.240	6.069	-0.040	1.000
	Sucrose 20 E10	-0.430	6.069	-0.071	1.000
	Trehalose 20 E10	-4.920	6.069	-0.811	0.993
	Sucrose 10 E9	-9.290	6.069	-1.531	0.854
	Trehalose 10 E9	3.590	6.069	0.592	0.999
	Sucrose 15 E9	-0.285	6.069	-0.047	1.000
	Trehalose 15 E9	2.175	6.069	0.358	1.000
	Sucrose 20 E9	2.305	6.069	0.380	1.000
	Trehalose 20 E9	4.055	6.069	0.668	0.998
Trehalose 10 E10	Sucrose 15 E10	-7.250	6.069	-1.195	0.942
	Trehalose 15 E10	-0.975	6.069	-0.161	1.000
	Sucrose 20 E10	-1.165	6.069	-0.192	1.000
	Trehalose 20 E10	-5.655	6.069	-0.932	0.983
	Sucrose 10 E9	-10.025	6.069	-1.652	0.816
	Trehalose 10 E9	2.855	6.069	0.470	1.000
	Sucrose 15 E9	-1.020	6.069	-0.168	1.000
	Trehalose 15 E9	1.440	6.069	0.237	1.000
	Sucrose 20 E9	1.570	6.069	0.259	1.000
	Trehalose 20 E9	3.320	6.069	0.547	1.000
Sucrose 15 E10	Trehalose 15 E10	6.275	6.069	1.034	0.970
	Sucrose 20 E10	6.085	6.069	1.003	0.975
	Trehalose 20 E10	1.595	6.069	0.263	1.000
	Sucrose 10 E9	-2.775	6.069	-0.457	1.000
	Trehalose 10 E9	10.105	6.069	1.665	0.812
	Sucrose 15 E9	6.230	6.069	1.027	0.971
	Trehalose 15 E9	8.690	6.069	1.432	0.883
	Sucrose 20 E9	8.820	6.069	1.453	0.877
	Trehalose 20 E9	10.570	6.069	1.742	0.788
Trehalose 15 E10	Sucrose 20 E10	-0.190	6.069	-0.031	1.000
	Trehalose 20 E10	-4.680	6.069	-0.771	0.995
	Sucrose 10 E9	-9.050	6.069	-1.491	0.866
	Trehalose 10 E9	3.830	6.069	0.631	0.999
	Sucrose 15 E9	-0.045	6.069	-0.007	1.000
	Trehalose 15 E9	2.415	6.069	0.398	1.000
	Sucrose 20 E9	2.545	6.069	0.419	1.000
	Trehalose 20 E9	4.295	6.069	0.708	0.997
Sucrose 20 E10	Trehalose 20 E10	-4.490	6.069	-0.740	0.996
	Sucrose 10 E9	-8.860	6.069	-1.460	0.875
	Trehalose 10 E9	4.020	6.069	0.662	0.998

**Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration \* Bacterial concentration**

		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
Trehalose 20 E10	Sucrose 15 E9	0.145	6.069	0.024	1.000
	Trehalose 15 E9	2.605	6.069	0.429	1.000
	Sucrose 20 E9	2.735	6.069	0.451	1.000
	Trehalose 20 E9	4.485	6.069	0.739	0.996
	Sucrose 10 E9	-4.370	6.069	-0.720	0.997
	Trehalose 10 E9	8.510	6.069	1.402	0.891
Sucrose 10 E9	Sucrose 15 E9	4.635	6.069	0.764	0.995
	Trehalose 15 E9	7.095	6.069	1.169	0.947
	Sucrose 20 E9	7.225	6.069	1.191	0.943
	Trehalose 20 E9	8.975	6.069	1.479	0.869
	Trehalose 10 E9	12.880	6.069	2.122	0.667
	Sucrose 15 E9	9.005	6.069	1.484	0.868
Trehalose 10 E9	Trehalose 15 E9	11.465	6.069	1.889	0.740
	Sucrose 20 E9	11.595	6.069	1.911	0.733
	Trehalose 20 E9	13.345	6.069	2.199	0.644
	Sucrose 15 E9	-3.875	6.069	-0.639	0.999
	Trehalose 15 E9	-1.415	6.069	-0.233	1.000
	Sucrose 20 E9	-1.285	6.069	-0.212	1.000
Sucrose 15 E9	Trehalose 20 E9	0.465	6.069	0.077	1.000
	Trehalose 15 E9	2.460	6.069	0.405	1.000
	Sucrose 20 E9	2.590	6.069	0.427	1.000
	Trehalose 20 E9	4.340	6.069	0.715	0.997
Trehalose 15 E9	Sucrose 20 E9	0.130	6.069	0.021	1.000
	Trehalose 20 E9	1.880	6.069	0.310	1.000
Sucrose 20 E9	Trehalose 20 E9	1.750	6.069	0.288	1.000

*Note.* P-value adjusted for comparing a family of 12

*Note.* Results are averaged over the levels of: Annealing

**Table S7.** Tukey post-hoc tests: effects and interactions of the factors on cake appearance of freeze-dried R2LC

## Post Hoc Tests-Cake appearance

### Standard

#### Post Hoc Comparisons - Lyoprotectant

		Mean Difference	SE	t	p <sub>Tukey</sub>
Sucrose	Trehalose	0.050	0.169	0.295	0.768

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Annealing

		Mean Difference	SE	t	p <sub>Tukey</sub>
No	Yes	-0.050	0.169	-0.295	0.768

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Lyoprotectant concentration

		Mean Difference	SE	t	p <sub>Tukey</sub>
10	15	0.125	0.207	0.602	0.819
	20	-0.975	0.207	-4.699	< .001 ***
15	20	-1.100	0.207	-5.301	< .001 ***

\*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial concentration

#### Post Hoc Comparisons - Bacterial concentration

		Mean Difference	SE	t	p <sub>Tukey</sub>
E10	E9	-0.350	0.169	-2.066	0.041 *

\* p < .05

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration

#### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	p <sub>Tukey</sub>
Sucrose No	Trehalose No	0.367	0.240	1.530	0.423
	Sucrose Yes	0.267	0.240	1.113	0.682
	Trehalose Yes	-1.228×10 <sup>-15</sup>	0.240	-5.126×10 <sup>-15</sup>	1.000
Trehalose No	Sucrose Yes	-0.100	0.240	-0.417	0.975
	Trehalose Yes	-0.367	0.240	-1.530	0.423

### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose Yes	Trehalose Yes	-0.267	0.240	-1.113	0.682

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose 10	Trehalose 10	-0.250	0.293	-0.852	0.957
	Sucrose 15	0.150	0.293	0.511	0.996
	Trehalose 15	-0.150	0.293	-0.511	0.996
	Sucrose 20	-1.450	0.293	-4.941	< .001 ***
	Trehalose 20	-0.750	0.293	-2.556	0.118
Trehalose 10	Sucrose 15	0.400	0.293	1.363	0.749
	Trehalose 15	0.100	0.293	0.341	0.999
	Sucrose 20	-1.200	0.293	-4.090	0.001 **
	Trehalose 20	-0.500	0.293	-1.704	0.532
Sucrose 15	Trehalose 15	-0.300	0.293	-1.022	0.909
	Sucrose 20	-1.600	0.293	-5.453	< .001 ***
	Trehalose 20	-0.900	0.293	-3.067	0.032 *
Trehalose 15	Sucrose 20	-1.300	0.293	-4.430	< .001 ***
	Trehalose 20	-0.600	0.293	-2.045	0.325
Sucrose 20	Trehalose 20	0.700	0.293	2.386	0.171

*Note.* Results are averaged over the levels of: Annealing, Bacterial concentration

*Note.* P-value adjusted for comparing a family of 6

\* p < .05, \*\* p < .01, \*\*\* p < .001

### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose E10	Trehalose E10	0.333	0.240	1.391	0.508
	Sucrose E9	-0.067	0.240	-0.278	0.992
	Trehalose E9	-0.300	0.240	-1.252	0.595
Trehalose E10	Sucrose E9	-0.400	0.240	-1.670	0.345
	Trehalose E9	-0.633	0.240	-2.643	0.046 *
Sucrose E9	Trehalose E9	-0.233	0.240	-0.974	0.765

\* p < .05

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration

### Post Hoc Comparisons - Annealing \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
No 10	Yes 10	0.150	0.293	0.511	0.996

### Post Hoc Comparisons - Annealing \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
Yes 10	No 15	0.100	0.293	0.341	0.999
	Yes 15	0.300	0.293	1.022	0.909
	No 20	-0.650	0.293	-2.215	0.240
	Yes 20	-1.150	0.293	-3.919	0.002 **
	No 15	-0.050	0.293	-0.170	1.000
	Yes 15	0.150	0.293	0.511	0.996
	No 20	-0.800	0.293	-2.726	0.079
	Yes 20	-1.300	0.293	-4.430	< .001 ***
No 15	Yes 15	0.200	0.293	0.682	0.984
	No 20	-0.750	0.293	-2.556	0.118
	Yes 20	-1.250	0.293	-4.260	< .001 ***
Yes 15	No 20	-0.950	0.293	-3.238	0.020 *
	Yes 20	-1.450	0.293	-4.941	< .001 ***
No 20	Yes 20	-0.500	0.293	-1.704	0.532

*Note.* Results are averaged over the levels of: Lyoprotectant, Bacterial concentration

*Note.* P-value adjusted for comparing a family of 6

\* p < .05, \*\* p < .01, \*\*\* p < .001

### Post Hoc Comparisons - Annealing \* Bacterial concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
No E10	Yes E10	6.939×10 <sup>-18</sup>	0.240	2.896×10 <sup>-17</sup>	1.000
	No E9	-0.300	0.240	-1.252	0.595
	Yes E9	-0.400	0.240	-1.670	0.345
Yes E10	No E9	-0.300	0.240	-1.252	0.595
	Yes E9	-0.400	0.240	-1.670	0.345
No E9	Yes E9	-0.100	0.240	-0.417	0.975

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration

### Post Hoc Comparisons - Lyoprotectant concentration \* Bacterial concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
10 E10	15 E10	0.200	0.293	0.682	0.984
	20 E10	-1.050	0.293	-3.578	0.007 **
	10 E9	-0.350	0.293	-1.193	0.839
	15 E9	-0.300	0.293	-1.022	0.909
	20 E9	-1.250	0.293	-4.260	< .001 ***
15 E10	20 E10	-1.250	0.293	-4.260	< .001 ***
	10 E9	-0.550	0.293	-1.874	0.424
	15 E9	-0.500	0.293	-1.704	0.532
	20 E9	-1.450	0.293	-4.941	< .001 ***
20 E10	10 E9	0.700	0.293	2.386	0.171
	15 E9	0.750	0.293	2.556	0.118

**Post Hoc Comparisons - Lyoprotectant concentration \* Bacterial concentration**

		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>P<sub>Tukey</sub></b>
	20 E9	-0.200	0.293	-0.682	0.984
10 E9	15 E9	0.050	0.293	0.170	1.000
	20 E9	-0.900	0.293	-3.067	0.032 *
15 E9	20 E9	-0.950	0.293	-3.238	0.020 *

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing

*Note.* P-value adjusted for comparing a family of 6

\*  $p < .05$ , \*\*  $p < .01$ , \*\*\*  $p < .001$

**Table S8.** Accelerated stability- Tukey post-hoc tests: effects and interactions of the factors on water content of freeze-dried R2LC

## Post Hoc Tests- Accelerated storage-water content

### Standard

#### Post Hoc Comparisons - Lyoprotectant

		Mean Difference	SE	t	Cohen's d	p <sub>tukey</sub>
Sucrose	Trehalose	0.198	0.141	1.404	0.234	0.163

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration (%), Bacterial Concentration

#### Post Hoc Comparisons - Annealing

		Mean Difference	SE	t	Cohen's d	p <sub>tukey</sub>
No	Yes	0.010	0.141	0.072	0.012	0.943

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration (%), Bacterial Concentration

#### Post Hoc Comparisons - Lyoprotectant concentration (%)

		Mean Difference	SE	t	Cohen's d	p <sub>tukey</sub>
10	15	-0.063	0.173	-0.364	-0.074	0.929
	20	-1.341	0.173	-7.743	-1.581	< .001 ***
15	20	-1.278	0.173	-7.379	-1.506	< .001 ***

\*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial Concentration

#### Post Hoc Comparisons - Bacterial Concentration

		Mean Difference	SE	t	Cohen's d	p <sub>tukey</sub>
e10	e9	-1.125	0.141	-7.955	-1.326	< .001 ***

\*\*\* p < .001

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration (%)

#### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	Cohen's d	p <sub>tukey</sub>
Sucrose No	Trehalose No	-0.355	0.200	-1.777	-0.419	0.289
	Sucrose Yes	-0.544	0.200	-2.718	-0.641	0.037 *
	Trehalose Yes	0.209	0.200	1.043	0.246	0.724
Trehalose No	Sucrose Yes	-0.188	0.200	-0.942	-0.222	0.782
	Trehalose Yes	0.564	0.200	2.820	0.665	0.028 *



### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	Cohen's d	p <sub>tukey</sub>
Sucrose Yes	Trehalose Yes	0.752	0.200	3.761	0.887	0.001 **

\* p < .05, \*\* p < .01

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration (%), Bacterial Concentration

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration (%)

		Mean Difference	SE	t	Cohen's d	p <sub>tukey</sub>
Sucrose 10	Trehalose 10	-0.209	0.245	-0.852	-0.246	0.957
	Sucrose 15	-0.318	0.245	-1.296	-0.374	0.787
	Trehalose 15	-0.018	0.245	-0.071	-0.021	1.000
	Sucrose 20	-1.698	0.245	-6.931	-2.001	< .001 ***
	Trehalose 20	-1.193	0.245	-4.872	-1.406	< .001 ***
Trehalose 10	Sucrose 15	-0.109	0.245	-0.444	-0.128	0.998
	Trehalose 15	0.191	0.245	0.781	0.225	0.970
	Sucrose 20	-1.489	0.245	-6.078	-1.755	< .001 ***
	Trehalose 20	-0.985	0.245	-4.020	-1.160	0.001 **
Sucrose 15	Trehalose 15	0.300	0.245	1.225	0.354	0.824
	Sucrose 20	-1.380	0.245	-5.634	-1.626	< .001 ***
	Trehalose 20	-0.876	0.245	-3.576	-1.032	0.006 **
Trehalose 15	Sucrose 20	-1.680	0.245	-6.859	-1.980	< .001 ***
	Trehalose 20	-1.176	0.245	-4.801	-1.386	< .001 ***
Sucrose 20	Trehalose 20	0.504	0.245	2.058	0.594	0.316

\* p < .05, \*\* p < .01, \*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 6

*Note.* Results are averaged over the levels of: Annealing, Bacterial Concentration

### Post Hoc Comparisons - Annealing \* Lyoprotectant concentration (%)

		Mean Difference	SE	t	Cohen's d	p <sub>tukey</sub>
No 10	Yes 10	-0.189	0.245	-0.771	-0.222	0.972
	No 15	-0.204	0.245	-0.832	-0.240	0.961
	Yes 15	-0.111	0.245	-0.454	-0.131	0.998
	No 20	-1.499	0.245	-6.119	-1.766	< .001 ***
	Yes 20	-1.372	0.245	-5.602	-1.617	< .001 ***
Yes 10	No 15	-0.015	0.245	-0.061	-0.018	1.000
	Yes 15	0.077	0.245	0.316	0.091	1.000
	No 20	-1.310	0.245	-5.349	-1.544	< .001 ***
	Yes 20	-1.183	0.245	-4.831	-1.395	< .001 ***
No 15	Yes 15	0.093	0.245	0.378	0.109	0.999
	No 20	-1.295	0.245	-5.287	-1.526	< .001 ***
	Yes 20	-1.168	0.245	-4.770	-1.377	< .001 ***
Yes 15	No 20	-1.388	0.245	-5.665	-1.635	< .001 ***

### Post Hoc Comparisons - Annealing \* Lyoprotectant concentration (%)

		Mean Difference	SE	t	Cohen's d	p <sub>Tukey</sub>
	Yes 20	-1.261	0.245	-5.148	-1.486	< .001 ***
No 20	Yes 20	0.127	0.245	0.517	0.149	0.995

\*\*\* p < .001

Note. P-value adjusted for comparing a family of 6

Note. Results are averaged over the levels of: Lyoprotectant, Bacterial Concentration

### Post Hoc Comparisons - Lyoprotectant \* Bacterial Concentration

		Mean Difference	SE	t	Cohen's d	p <sub>Tukey</sub>
Sucrose e10	Trehalose e10	0.112	0.200	0.560	0.132	0.944
	Sucrose e9	-1.211	0.200	-6.057	-1.428	< .001 ***
	Trehalose e9	-0.926	0.200	-4.632	-1.092	< .001 ***
Trehalose e10	Sucrose e9	-1.323	0.200	-6.617	-1.560	< .001 ***
	Trehalose e9	-1.038	0.200	-5.192	-1.224	< .001 ***
Sucrose e9	Trehalose e9	0.285	0.200	1.425	0.336	0.486

\*\*\* p < .001

Note. P-value adjusted for comparing a family of 4

Note. Results are averaged over the levels of: Annealing, Lyoprotectant concentration (%)

### Post Hoc Comparisons - Annealing \* Bacterial Concentration

		Mean Difference	SE	t	Cohen's d	p <sub>Tukey</sub>
No e10	Yes e10	-0.252	0.200	-1.263	-0.298	0.588
	No e9	-1.387	0.200	-6.938	-1.635	< .001 ***
	Yes e9	-1.115	0.200	-5.574	-1.314	< .001 ***
Yes e10	No e9	-1.135	0.200	-5.676	-1.338	< .001 ***
	Yes e9	-0.862	0.200	-4.312	-1.016	< .001 ***
No e9	Yes e9	0.273	0.200	1.364	0.322	0.524

\*\*\* p < .001

Note. P-value adjusted for comparing a family of 4

Note. Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration (%)

### Post Hoc Comparisons - Lyoprotectant concentration (%) \* Bacterial Concentration

		Mean Difference	SE	t	Cohen's d	p <sub>Tukey</sub>
10 e10	15 e10	0.108	0.245	0.442	0.128	0.998
	20 e10	-0.603	0.245	-2.463	-0.711	0.143
	10 e9	-0.519	0.245	-2.118	-0.611	0.285
	15 e9	-0.753	0.245	-3.076	-0.888	0.030 *
	20 e9	-2.598	0.245	-10.605	-3.061	< .001 ***
15 e10	20 e10	-0.712	0.245	-2.906	-0.839	0.048 *
	10 e9	-0.627	0.245	-2.560	-0.739	0.115
	15 e9	-0.862	0.245	-3.518	-1.016	0.008 **

**Post Hoc Comparisons - Lyoprotectant concentration (%) \* Bacterial Concentration**

		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>Cohen's d</b>	<b>p<sub>Tukey</sub></b>
	20 e9	-2.706	0.245	-11.048	-3.189	< .001 ***
20 e10	10 e9	0.085	0.245	0.345	0.100	0.999
	15 e9	-0.150	0.245	-0.612	-0.177	0.990
	20 e9	-1.994	0.245	-8.142	-2.350	< .001 ***
10 e9	15 e9	-0.235	0.245	-0.958	-0.276	0.930
	20 e9	-2.079	0.245	-8.487	-2.450	< .001 ***
15 e9	20 e9	-1.844	0.245	-7.529	-2.174	< .001 ***

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing

*Note.* P-value adjusted for comparing a family of 6

\* p < .05, \*\* p < .01, \*\*\* p < .001

**Table S9.** Post-hoc test to evaluate the effect of all variables on freeze-drying survival of freeze-dried R2LC.

## Post Hoc Tests- Freeze-dried Survival

### Standard

#### Post Hoc Comparisons - Lyoprotectant

		Mean Difference	SE	t	p <sub>Tukey</sub>
Sucrose	Trehalose	25.420	3.628	7.007	< .001

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Annealing

		Mean Difference	SE	t	p <sub>Tukey</sub>
No	Yes	13.073	3.628	3.604	< .001

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Lyoprotectant concentration

		Mean Difference	SE	t	p <sub>Tukey</sub>
10	15	-11.131	4.443	-2.505	0.044
	20	-9.136	4.443	-2.056	0.114
15	20	1.995	4.443	0.449	0.895

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial concentration

#### Post Hoc Comparisons - Bacterial concentration

		Mean Difference	SE	t	p <sub>Tukey</sub>
E10	E9	0.041	3.628	0.011	0.991

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration

#### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	p <sub>Tukey</sub>
Sucrose No	Trehalose No	8.298	5.131	1.617	0.382
	Sucrose Yes	-4.049	5.131	-0.789	0.859
	Trehalose Yes	38.494	5.131	7.503	< .001
Trehalose No	Sucrose Yes	-12.347	5.131	-2.407	0.094
	Trehalose Yes	30.195	5.131	5.885	< .001

### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose Yes	Trehalose Yes	42.542	5.131	8.292	< .001

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose 10	Trehalose 10	28.312	6.284	4.506	< .001
	Sucrose 15	-16.143	6.284	-2.569	0.132
	Trehalose 15	22.193	6.284	3.532	0.014
	Sucrose 20	0.214	6.284	0.034	1.000
	Trehalose 20	9.827	6.284	1.564	0.627
Trehalose 10	Sucrose 15	-44.455	6.284	-7.075	< .001
	Trehalose 15	-6.119	6.284	-0.974	0.923
	Sucrose 20	-28.098	6.284	-4.472	0.001
	Trehalose 20	-18.486	6.284	-2.942	0.059
Sucrose 15	Trehalose 15	38.336	6.284	6.101	< .001
	Sucrose 20	16.357	6.284	2.603	0.123
	Trehalose 20	25.970	6.284	4.133	0.003
Trehalose 15	Sucrose 20	-21.979	6.284	-3.498	0.015
	Trehalose 20	-12.367	6.284	-1.968	0.380
Sucrose 20	Trehalose 20	9.613	6.284	1.530	0.648

*Note.* P-value adjusted for comparing a family of 6

*Note.* Results are averaged over the levels of: Annealing, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose E10	Trehalose E10	18.943	5.131	3.692	0.004
	Sucrose E9	-6.437	5.131	-1.255	0.597
	Trehalose E9	25.462	5.131	4.963	< .001
Trehalose E10	Sucrose E9	-25.379	5.131	-4.947	< .001
	Trehalose E9	6.519	5.131	1.271	0.587
Sucrose E9	Trehalose E9	31.898	5.131	6.217	< .001

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration

**Table S10.** Post-hoc test to evaluate the effect of all variables on freeze-drying metabolic activity of freeze-dried R2LC

## Post Hoc Tests- Metabolic activity

### Standard

#### Post Hoc Comparisons - Lyoprotectant

		Mean Difference	SE	t	ptukey
Sucrose	Trehalose	0.483	0.011	43.608	< .001

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Annealing

		Mean Difference	SE	t	ptukey
No	Yes	-0.130	0.011	-11.729	< .001

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Lyoprotectant concentration

		Mean Difference	SE	t	ptukey
10	15	-0.028	0.014	-2.072	0.110
	20	-0.015	0.014	-1.105	0.517
15	20	0.013	0.014	0.967	0.602

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial concentration

#### Post Hoc Comparisons - Bacterial concentration

		Mean Difference	SE	t	ptukey
E10	E9	0.386	0.011	34.811	< .001

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration

#### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	ptukey
Sucrose No	Trehalose No	0.356	0.016	22.701	< .001
	Sucrose Yes	-0.258	0.016	-16.428	< .001
	Trehalose Yes	0.353	0.016	22.542	< .001
Trehalose No	Sucrose Yes	-0.613	0.016	-39.129	< .001

### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	P <sub>Tukey</sub>
Sucrose Yes	Trehalose Yes	-0.003	0.016	-0.159	0.999
	Trehalose Yes	0.611	0.016	38.970	< .001

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>Tukey</sub>
Sucrose 10	Trehalose 10	0.474	0.019	24.678	< .001
	Sucrose 15	-0.041	0.019	-2.149	0.287
	Trehalose 15	0.459	0.019	23.896	< .001
	Sucrose 20	-0.016	0.019	-0.846	0.956
Trehalose 10	Trehalose 20	0.460	0.019	23.962	< .001
	Sucrose 15	-0.515	0.019	-26.826	< .001
	Trehalose 15	-0.015	0.019	-0.781	0.969
	Sucrose 20	-0.490	0.019	-25.524	< .001
Sucrose 15	Trehalose 20	-0.014	0.019	-0.716	0.979
	Trehalose 15	0.500	0.019	26.045	< .001
	Sucrose 20	0.025	0.019	1.302	0.782
	Trehalose 20	0.501	0.019	26.110	< .001
Trehalose 15	Sucrose 20	-0.475	0.019	-24.743	< .001
	Trehalose 20	0.001	0.019	0.065	1.000
Sucrose 20	Trehalose 20	0.476	0.019	24.808	< .001

*Note.* P-value adjusted for comparing a family of 6

*Note.* Results are averaged over the levels of: Annealing, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	P <sub>Tukey</sub>
Sucrose E10	Trehalose E10	0.487	0.016	31.048	< .001
	Sucrose E9	0.389	0.016	24.828	< .001
	Trehalose E9	0.869	0.016	55.450	< .001
Trehalose E10	Sucrose E9	-0.098	0.016	-6.220	< .001
	Trehalose E9	0.383	0.016	24.402	< .001
Sucrose E9	Trehalose E9	0.480	0.016	30.623	< .001

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration

**Table S11.** Post-hoc test to evaluate the effect of accelerated storage stability (week 2) on survival. \*p<0.05, \*\*p<0.01, and \*\*\*p<0.001

## Post Hoc Tests- Accelerated stability survival 2

### Standard

#### Post Hoc Comparisons - Lyoprotectant

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose	Trehalose	15.106	3.424	4.412	< .001

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Annealing

		Mean Difference	SE	t	p <sub>tukey</sub>
No	Yes	-6.080	3.424	-1.776	0.084

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Lyoprotectant concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
10	15	-8.720	4.193	-2.080	0.109
	20	-2.298	4.193	-0.548	0.848
15	20	6.422	4.193	1.532	0.289

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial concentration

#### Post Hoc Comparisons - Bacterial concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
E10	E9	7.989	3.424	2.333	0.025

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration

#### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose No	Trehalose No	5.468	4.842	1.129	0.674
	Sucrose Yes	-15.718	4.842	-3.247	0.013
	Trehalose Yes	9.026	4.842	1.864	0.262
Trehalose No	Sucrose Yes	-21.187	4.842	-4.376	< .001



### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	P <sub>tukey</sub>
	Trehalose Yes	3.557	4.842	0.735	0.882
Sucrose Yes	Trehalose Yes	24.744	4.842	5.111	< .001

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose 10	Trehalose 10	18.390	5.930	3.101	0.041
	Sucrose 15	-11.708	5.930	-1.974	0.377
	Trehalose 15	12.657	5.930	2.135	0.294
	Sucrose 20	5.615	5.930	0.947	0.931
	Trehalose 20	8.179	5.930	1.379	0.739
Trehalose 10	Sucrose 15	-30.098	5.930	-5.076	< .001
	Trehalose 15	-5.732	5.930	-0.967	0.925
	Sucrose 20	-12.775	5.930	-2.154	0.284
	Trehalose 20	-10.211	5.930	-1.722	0.527
Sucrose 15	Trehalose 15	24.365	5.930	4.109	0.003
	Sucrose 20	17.323	5.930	2.921	0.062
	Trehalose 20	19.886	5.930	3.354	0.022
Trehalose 15	Sucrose 20	-7.042	5.930	-1.188	0.840
	Trehalose 20	-4.479	5.930	-0.755	0.973
Sucrose 20	Trehalose 20	2.564	5.930	0.432	0.998

*Note.* P-value adjusted for comparing a family of 6

*Note.* Results are averaged over the levels of: Annealing, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	P <sub>tukey</sub>
Sucrose E10	Trehalose E10	6.402	4.842	1.322	0.555
	Sucrose E9	-0.715	4.842	-0.148	0.999
	Trehalose E9	23.095	4.842	4.770	< .001
Trehalose E10	Sucrose E9	-7.117	4.842	-1.470	0.466
	Trehalose E9	16.692	4.842	3.448	0.008
Sucrose E9	Trehalose E9	23.810	4.842	4.918	< .001

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration

**Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration \* Bacterial concentration**

		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>P<sub>tukey</sub></b>
Sucrose 10 E10	Trehalose 10 E10	-0.720	7.282	-0.099	1.000
	Sucrose 15 E10	-22.713	7.282	-3.119	0.127
	Trehalose 15 E10	-12.293	7.282	-1.688	0.859
	Sucrose 20 E10	-25.883	7.282	-3.554	0.050
	Trehalose 20 E10	-16.375	7.282	-2.249	0.531
	Sucrose 10 E9	-29.050	7.282	-3.989	0.018 *
	Trehalose 10 E9	8.450	7.282	1.160	0.988
	Sucrose 15 E9	-29.753	7.282	-4.086	0.014 *
	Trehalose 15 E9	8.557	7.282	1.175	0.987
	Sucrose 20 E9	8.062	7.282	1.107	0.992
	Trehalose 20 E9	3.682	7.282	0.506	1.000
Trehalose 10 E10	Sucrose 15 E10	-21.992	7.282	-3.020	0.155
	Trehalose 15 E10	-11.573	7.282	-1.589	0.899
	Sucrose 20 E10	-25.162	7.282	-3.455	0.063
	Trehalose 20 E10	-15.655	7.282	-2.150	0.595
	Sucrose 10 E9	-28.330	7.282	-3.890	0.023 *
	Trehalose 10 E9	9.170	7.282	1.259	0.978
	Sucrose 15 E9	-29.033	7.282	-3.987	0.018 *
	Trehalose 15 E9	9.278	7.282	1.274	0.976
	Sucrose 20 E9	8.783	7.282	1.206	0.984
	Trehalose 20 E9	4.403	7.282	0.605	1.000
Sucrose 15 E10	Trehalose 15 E10	10.420	7.282	1.431	0.947
	Sucrose 20 E10	-3.170	7.282	-0.435	1.000
	Trehalose 20 E10	6.338	7.282	0.870	0.999
	Sucrose 10 E9	-6.337	7.282	-0.870	0.999
	Trehalose 10 E9	31.162	7.282	4.279	0.009 **
	Sucrose 15 E9	-7.040	7.282	-0.967	0.997
	Trehalose 15 E9	31.270	7.282	4.294	0.008 **
	Sucrose 20 E9	30.775	7.282	4.226	0.010 *
	Trehalose 20 E9	26.395	7.282	3.625	0.043 *
Trehalose 15 E10	Sucrose 20 E10	-13.590	7.282	-1.866	0.769
	Trehalose 20 E10	-4.082	7.282	-0.561	1.000
	Sucrose 10 E9	-16.757	7.282	-2.301	0.498
	Trehalose 10 E9	20.742	7.282	2.848	0.214
	Sucrose 15 E9	-17.460	7.282	-2.398	0.439
	Trehalose 15 E9	20.850	7.282	2.863	0.208
	Sucrose 20 E9	20.355	7.282	2.795	0.235
	Trehalose 20 E9	15.975	7.282	2.194	0.567
Sucrose 20 E10	Trehalose 20 E10	9.507	7.282	1.306	0.971
	Sucrose 10 E9	-3.167	7.282	-0.435	1.000
	Trehalose 10 E9	34.332	7.282	4.715	0.003 **

**Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration \* Bacterial concentration**

		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
	Sucrose 15 E9	-3.870	7.282	-0.531	1.000
	Trehalose 15 E9	34.440	7.282	4.729	0.003 **
	Sucrose 20 E9	33.945	7.282	4.662	0.003 **
	Trehalose 20 E9	29.565	7.282	4.060	0.015 *
Trehalose 20 E10	Sucrose 10 E9	-12.675	7.282	-1.741	0.835
	Trehalose 10 E9	24.825	7.282	3.409	0.069
	Sucrose 15 E9	-13.378	7.282	-1.837	0.785
	Trehalose 15 E9	24.932	7.282	3.424	0.067
	Sucrose 20 E9	24.438	7.282	3.356	0.078
	Trehalose 20 E9	20.057	7.282	2.754	0.252
Sucrose 10 E9	Trehalose 10 E9	37.500	7.282	5.150	< .001 ***
	Sucrose 15 E9	-0.703	7.282	-0.096	1.000
	Trehalose 15 E9	37.607	7.282	5.164	< .001 ***
	Sucrose 20 E9	37.112	7.282	5.096	0.001 **
	Trehalose 20 E9	32.732	7.282	4.495	0.005 **
	Sucrose 15 E9	-38.203	7.282	-5.246	< .001 ***
Trehalose 10 E9	Trehalose 15 E9	0.108	7.282	0.015	1.000
	Sucrose 20 E9	-0.387	7.282	-0.053	1.000
	Trehalose 20 E9	-4.767	7.282	-0.655	1.000
	Sucrose 15 E9	38.310	7.282	5.261	< .001 ***
	Sucrose 20 E9	37.815	7.282	5.193	< .001 ***
	Trehalose 20 E9	33.435	7.282	4.591	0.004 **
Trehalose 15 E9	Sucrose 20 E9	-0.495	7.282	-0.068	1.000
	Trehalose 20 E9	-4.875	7.282	-0.669	1.000
Sucrose 20 E9	Trehalose 20 E9	-4.380	7.282	-0.601	1.000

\* p < .05, \*\* p < .01, \*\*\* p < .001

*Note.* P-value adjusted for comparing a family of 12

*Note.* Results are averaged over the levels of: Annealing

**Table S12.** Post-hoc test to evaluate the effect of accelerated storage stability (week 2) on metabolic activity. \*p<0.05, \*\*p<0.01, and \*\*\*p<0.001

## Post Hoc Tests-Accelerated storage metabolic activity week 2

### Standard

#### Post Hoc Comparisons - Lyoprotectant

		Mean Difference	SE	t	ptukey
Sucrose	Trehalose	0.099	0.041	2.444	0.033

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Annealing

		Mean Difference	SE	t	ptukey
No	Yes	-0.043	0.041	-1.047	0.317

*Note.* Results are averaged over the levels of: Lyoprotectant, Lyoprotectant concentration, Bacterial concentration

#### Post Hoc Comparisons - Lyoprotectant concentration

		Mean Difference	SE	t	ptukey
10	15	-0.114	0.050	-2.289	0.100
	20	-0.071	0.050	-1.434	0.358
15	20	0.043	0.050	0.855	0.678

*Note.* P-value adjusted for comparing a family of 3

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Bacterial concentration

#### Post Hoc Comparisons - Bacterial concentration

		Mean Difference	SE	t	ptukey
E10	E9	0.272	0.041	6.715	< .001

*Note.* Results are averaged over the levels of: Lyoprotectant, Annealing, Lyoprotectant concentration

#### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	ptukey
Sucrose No	Trehalose No	0.063	0.057	1.104	0.695
	Sucrose Yes	-0.078	0.057	-1.365	0.544
	Trehalose Yes	0.057	0.057	0.987	0.759
Trehalose No	Sucrose Yes	-0.142	0.057	-2.468	0.121

### Post Hoc Comparisons - Lyoprotectant \* Annealing

		Mean Difference	SE	t	p <sub>tukey</sub>
	Trehalose Yes	-0.007	0.057	-0.116	0.999
Sucrose Yes	Trehalose Yes	0.135	0.057	2.352	0.145

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Lyoprotectant concentration, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose 10	Trehalose 10	0.043	0.070	0.605	0.988
	Sucrose 15	-0.190	0.070	-2.703	0.151
	Trehalose 15	0.005	0.070	0.071	1.000
	Sucrose 20	-0.080	0.070	-1.138	0.856
	Trehalose 20	-0.020	0.070	-0.285	1.000
Trehalose 10	Sucrose 15	-0.232	0.070	-3.308	0.059
	Trehalose 15	-0.037	0.070	-0.534	0.993
	Sucrose 20	-0.122	0.070	-1.743	0.534
	Trehalose 20	-0.062	0.070	-0.889	0.941
Sucrose 15	Trehalose 15	0.195	0.070	2.774	0.136
	Sucrose 20	0.110	0.070	1.565	0.635
	Trehalose 20	0.170	0.070	2.419	0.230
Trehalose 15	Sucrose 20	-0.085	0.070	-1.209	0.824
	Trehalose 20	-0.025	0.070	-0.356	0.999
Sucrose 20	Trehalose 20	0.060	0.070	0.854	0.950

*Note.* P-value adjusted for comparing a family of 6

*Note.* Results are averaged over the levels of: Annealing, Bacterial concentration

### Post Hoc Comparisons - Lyoprotectant \* Bacterial concentration

		Mean Difference	SE	t	p <sub>tukey</sub>
Sucrose E10	Trehalose E10	0.182	0.057	3.165	0.039
	Sucrose E9	0.355	0.057	6.186	< .001
	Trehalose E9	0.372	0.057	6.476	< .001
Trehalose E10	Sucrose E9	0.173	0.057	3.020	0.049
	Trehalose E9	0.190	0.057	3.311	0.030
Sucrose E9	Trehalose E9	0.017	0.057	0.290	0.991

*Note.* P-value adjusted for comparing a family of 4

*Note.* Results are averaged over the levels of: Annealing, Lyoprotectant concentration

**Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration \* Bacterial concentration**

		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
Sucrose 10 E10	Trehalose 10 E10	-0.055	0.043	-1.280	0.944
	Sucrose 15 E10	-0.490	0.043	-11.405	0.004 **
	Trehalose 15 E10	-0.115	0.043	-2.677	0.435
	Sucrose 20 E10	-0.440	0.043	-10.241	0.006 **
	Trehalose 20 E10	-0.215	0.043	-5.004	0.082
	Sucrose 10 E9	-0.085	0.043	-1.978	0.700
	Trehalose 10 E9	0.055	0.043	1.280	0.944
	Sucrose 15 E9	0.025	0.043	0.582	1.000
	Trehalose 15 E9	0.040	0.043	0.931	0.992
	Sucrose 20 E9	0.195	0.043	4.539	0.111
	Trehalose 20 E9	0.090	0.043	2.095	0.652
Trehalose 10 E10	Sucrose 15 E10	-0.435	0.043	-10.125	0.007 **
	Trehalose 15 E10	-0.060	0.043	-1.397	0.915
	Sucrose 20 E10	-0.385	0.043	-8.961	0.010 *
	Trehalose 20 E10	-0.160	0.043	-3.724	0.199
	Sucrose 10 E9	-0.030	0.043	-0.698	0.999
	Trehalose 10 E9	0.110	0.043	2.560	0.474
	Sucrose 15 E9	0.080	0.043	1.862	0.748
	Trehalose 15 E9	0.095	0.043	2.211	0.604
	Sucrose 20 E9	0.250	0.043	5.819	0.050 *
	Trehalose 20 E9	0.145	0.043	3.375	0.257
Sucrose 15 E10	Trehalose 15 E10	0.375	0.043	8.728	0.012 *
	Sucrose 20 E10	0.050	0.043	1.164	0.966
	Trehalose 20 E10	0.275	0.043	6.401	0.036 *
	Sucrose 10 E9	0.405	0.043	9.427	0.009 **
	Trehalose 10 E9	0.545	0.043	12.685	0.003 **
	Sucrose 15 E9	0.515	0.043	11.987	0.003 **
	Trehalose 15 E9	0.530	0.043	12.336	0.003 **
	Sucrose 20 E9	0.685	0.043	15.944	0.001 **
	Trehalose 20 E9	0.580	0.043	13.500	0.002 **
Trehalose 15 E10	Sucrose 20 E10	-0.325	0.043	-7.565	0.020 *
	Trehalose 20 E10	-0.100	0.043	-2.328	0.559
	Sucrose 10 E9	0.030	0.043	0.698	0.999
	Trehalose 10 E9	0.170	0.043	3.957	0.168
	Sucrose 15 E9	0.140	0.043	3.259	0.281
	Trehalose 15 E9	0.155	0.043	3.608	0.216
	Sucrose 20 E9	0.310	0.043	7.215	0.023 *
	Trehalose 20 E9	0.205	0.043	4.772	0.095
Sucrose 20 E10	Trehalose 20 E10	0.225	0.043	5.237	0.071
	Sucrose 10 E9	0.355	0.043	8.263	0.014 *
	Trehalose 10 E9	0.495	0.043	11.521	0.004 **

**Post Hoc Comparisons - Lyoprotectant \* Lyoprotectant concentration \* Bacterial concentration**

		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>Tukey</sub></b>
	Sucrose 15 E9	0.465	0.043	10.823	0.005 **
	Trehalose 15 E9	0.480	0.043	11.172	0.005 **
	Sucrose 20 E9	0.635	0.043	14.780	0.002 **
	Trehalose 20 E9	0.530	0.043	12.336	0.003 **
Trehalose 20 E10	Sucrose 10 E9	0.130	0.043	3.026	0.335
	Trehalose 10 E9	0.270	0.043	6.284	0.038 *
	Sucrose 15 E9	0.240	0.043	5.586	0.057
	Trehalose 15 E9	0.255	0.043	5.935	0.046 *
	Sucrose 20 E9	0.410	0.043	9.543	0.008 **
	Trehalose 20 E9	0.305	0.043	7.099	0.025 *
Sucrose 10 E9	Trehalose 10 E9	0.140	0.043	3.259	0.281
	Sucrose 15 E9	0.110	0.043	2.560	0.474
	Trehalose 15 E9	0.125	0.043	2.909	0.366
	Sucrose 20 E9	0.280	0.043	6.517	0.033 *
	Trehalose 20 E9	0.175	0.043	4.073	0.154
Trehalose 10 E9	Sucrose 15 E9	-0.030	0.043	-0.698	0.999
	Trehalose 15 E9	-0.015	0.043	-0.349	1.000
	Sucrose 20 E9	0.140	0.043	3.259	0.281
	Trehalose 20 E9	0.035	0.043	0.815	0.997
Sucrose 15 E9	Trehalose 15 E9	0.015	0.043	0.349	1.000
	Sucrose 20 E9	0.170	0.043	3.957	0.168
	Trehalose 20 E9	0.065	0.043	1.513	0.880
Trehalose 15 E9	Sucrose 20 E9	0.155	0.043	3.608	0.216
	Trehalose 20 E9	0.050	0.043	1.164	0.966
Sucrose 20 E9	Trehalose 20 E9	-0.105	0.043	-2.444	0.515

\* p < .05, \*\* p < .01

*Note.* P-value adjusted for comparing a family of 12

*Note.* Results are averaged over the levels of: Annealing