Fermentative behavior of *Candida zemplinina*

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Purpose of the study: to describe the fermentation behavior of different strains of *C. zemplinina* when inoculated in sterile grape must *with varying sugar concentrations*

Experimental approach: pure cultures of 6 strains of *C. zemplinina* were inoculated in sterile grape must with adjusted sugar concentration of 200, 210, 265, 311 and 330 g L^{-1}. Viable counts and chemical analysis of the musts was performed at regular time intervals in order to monitor the dynamics of the strains and the evolution of the alcoholic fermentation. The influence of two factors, namely initial sugar concentration and time of fermentation, on important chemical parameters was determined by applying the Central Composite Design (CCD) approach.

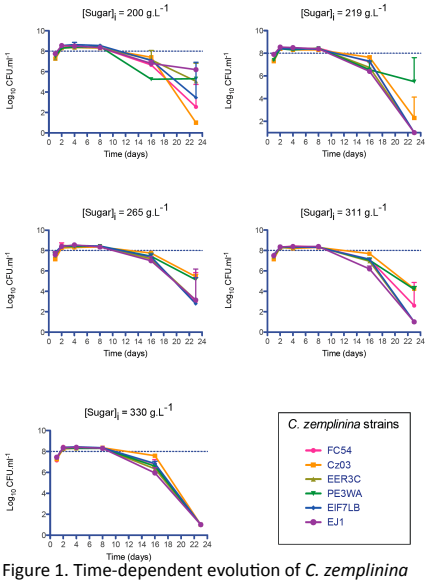

Figure 1. Time-dependent evolution of *C. zemplinina* populations. Yeasts were inoculated in pasteurized *Barbera* cv. must containing different initial sugar concentration.

Significant effect of initial sugar concentration on glucose consumption; inhibition of consumption at higher concentrations. Significant time effect of time as well

Significant effect of time on fructose consumption but no effect of initial sugar concentration

Table 1. Time and sugars effects on glucose and fructose concentration

Linear (L) and quadratic (Q) effects of sugar concentration and time on glucose concentration

	FC54		C703		EER3C		PE3WA		EIF7LB		EJ1	
Factor	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p
(1)[Sugar](L)	66.92	0.00	65.21	0.00	70.13	0.00	72.49	0.00	70.68	0.00	69.76	0.00
[Sugar](Q)	-6.93	0.06	-7.79	0.05	-4.45	0.35	-9.45	0.02	0.32	0.96	-6.99	0.07
(2)Time (L)	-44.90	0.00	-37.89	0.00	-48.92	0.00	-41.40	0.00	-47.38	0.00	-54.05	0.00
Time (Q)	2.52	0.00	20.30	0.00	36.07	0.00	25.72	0.00	33.45	0.00	28.85	0.00
1L by 2L	21.20	0.00	22.57	0.00	18.84	0.00	24.86	0.00	24.76	0.00	19.59	0.00

Red color indicate a significant effect

Linear (L) and quadratic (Q) effects of sugar concentration and time on fructose concentration

	FC54		C703		EER3C		PE3WA		EIF7LB		EJ1	
Factor	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p
(1)[Sugar](L)	12.01	0.08	11.49	0.06	11.12	0.04	13.34	0.06	12.64	0.06	12.41	0.08
[Sugar](Q)	-3.44	0.65	-2.39	0.72	-1.17	0.84	-3.38	0.67	-3.21	0.67	-3.67	0.64
(2)Time (L)	-76.84	0.00	-78.81	0.00	-81.16	0.00	-76.43	0.00	-77.06	0.00	-76.16	0.00
Time (Q)	58.41	0.00	59.54	0.00	60.80	0.00	58.26	0.00	58.67	0.00	58.56	0.00
1L by 2L	-21.73	0.03	-20.75	0.02	-19.31	0.01	-24.18	0.02	-23.49	0.02	-22.87	0.02

Red color indicate a significant effect

Significant effect of time on ethanol and glycerol production. With the exception of strains Cz03, EER3C and PE3WA, no effect of initial sugar concentration

Table 2. Time and sugars effects on ethanol and glycerol production

Linear (L) and quadratic (Q) effects of sugar concentration and time on ethanol content

	FC54		C703		EER3C		PE3WA		EIF7LB		EJ1	
Factor	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p
(1)[Sugar](L)	-0.34	0.23	-0.27	0.32	-0.21	0.36	-0.89	0.01	-0.63	0.17	-0.63	0.10
[Sugar](Q)	0.60	0.08	0.77	0.02	0.61	0.04	1.11	0.01	0.10	0.85	0.66	0.15
(2)Time (L)	6.79	0.00	6.47	0.00	7.12	0.00	6.31	0.00	6.89	0.00	6.36	0.00
Time (Q)	-4.43	0.00	-4.21	0.00	-4.77	0.00	-4.23	0.00	-4.80	0.00	-4.69	0.00
1L by 2L	-0.13	0.74	-0.17	0.66	0.56	0.10	-0.13	0.78	-0.30	0.63	0.27	0.60

Red color indicate a significant effect

Linear (L) and quadratic (Q) effects of sugar concentration and time on glycerol concentration

	FC54		C703		EER3C		PE3WA		EIF7LB		EJ1	
Factor	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p
(1)[Sugar](L)	0.47	0.40	0.88	0.11	0.57	0.25	0.48	0.41	0.53	0.44	0.49	0.46
[Sugar](Q)	1.02	0.14	1.10	0.10	1.30	0.04	1.15	0.10	0.52	0.52	0.66	0.40
(2)Time (L)	6.54	0.00	6.63	0.00	6.76	0.00	6.06	0.00	6.39	0.00	6.38	0.00
Time (Q)	-4.43	0.00	-4.19	0.00	-4.01	0.00	-3.84	0.00	-4.65	0.00	-4.22	0.00
1L by 2L	0.59	0.46	0.87	0.25	0.63	0.36	0.97	0.24	0.92	0.35	0.91	0.33

Red color indicate a significant effect

Significant effect of time on acetic acid production but no effect of initial sugar concentration

Significant effect of time and initial sugar concentration on malic acid consumption; consumption increases with time and is greater at lower sugar concentrations

Table 3. Time and sugars effects on acetic acid production and malic acid consumption

Linear (L) and quadratic (Q) effects of sugar concentration and time on acetic acid concentration

	FC54		C703		EER3C		PE3WA		EIF7LB		EJ1	
Factor	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p
(1)[Sugar](L)	0.03	0.28	0.03	0.28	0.04	0.17	0.01	0.63	0.03	0.37	0.06	0.03
[Sugar](Q)	-0.02	0.64	-0.04	0.30	-0.08	0.02	-0.04	0.22	-0.03	0.46	0.03	0.35
(2)Time (L)	0.23	0.00	0.28	0.00	0.36	0.00	0.29	0.00	0.26	0.00	0.26	0.00
Time (Q)	-0.16	0.00	-0.19	0.00	-0.28	0.00	-0.20	0.00	-0.20	0.00	-0.17	0.00
1L by 2L	0.01	0.77	-0.04	0.39	-0.04	0.33	-0.02	0.64	-0.02	0.58	0.03	0.44

Red color indicate a significant effect

Linear (L) and quadratic (Q) effects of sugar concentration and time on malic acid concentration

	FC54		C703		EER3C		PE3WA		EIF7LB		EJ1	
Factor	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p	Effect	p
(1)[Sugar](L)	0.37	0.00	0.36	0.00	0.33	0.00	0.35	0.00	0.34	0.00	0.24	0.00
[Sugar](Q)	-0.15	0.02	-0.02	0.73	-0.07	0.18	-0.05	0.27	-0.06	0.27	-0.08	0.07
(2)Time (L)	-0.79	0.00	-0.90	0.00	-0.71	0.00	-0.72	0.00	-0.76	0.00	-0.62	0.00
Time (Q)	0.28	0.00	0.13	0.05	0.32	0.00	0.30	0.00	0.22	0.00	0.24	0.00
1L by 2L	0.28	0.00	0.35	0.00	0.30	0.00	0.31	0.00	0.30	0.00	0.19	0.00

Red color indicate a significant effect

Main Conclusions

- The six *C. zemplinina* strains showed similar population kinetics and fermentation behavior
- The fructophilic character previously observed for *C. zemplinina* was confirmed
- Ethanol and glycerol were mainly produced in a time dependent manner and the production was not influenced by increased sugar concentration
- Acetic acid production increased over time. Similarly, malic acid was increasingly consumed with time and was greater at lower sugar concentrations
- Overall *C. zemplinina* demonstrated positive enological attributes in grape must with varying sugar concentrations