THERMAL INACTIVATION OF THE WINE SPOILAGE YEASTS *DEKKERA/BRETTANOMYCES*

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INTRODUCTION

Yeasts of the genus *Dekkera/Brettanomyces* are well known to be involved in the production of volatile phenols (4–ethyl-phenol and 4-ethyl-guaiacol) in wines imparting aroma defects usually described as “horse sweat”, “leather” and “animal”(Chatonnet P. *et al*., 1992). These yeasts are often associated with spoilage of barrel-aged wines due to insufficient wood sanitation (Chatonnet P *et al*., 1993). Thermal disinfection of barrels with hot water or steam is a commonly used sanitation process in the wine industry.

The heat resistance of three strains of this genus (*Dekkera anomala* PYCC 5153, *Dekkera bruxellensis* PYCC 4801 and *Dekkera/Brettanomyces* 093 strain isolated from contaminated wines) was evaluated at different temperatures (32,5-55ºC). The aim of this study was to establish regimes for the application of thermal control of *Dekkera/Brettanomyces* yeasts in wines and contaminated equipment.

MATERIALS AND METHODS

Initial thermal inactivation tests were performed in tartrate buffer (3,0 g L^{-1}) at various pH values (2,5-5,0) and ethanol concentrations (10-13% v/v). The effect of several phenolic compounds (ferulic, vanillic, caffeic and gallic acids at 500 mg L^{-1}) was also

tested. Subsequent tests were performed in sterile-filtered red wines. Cultures were grown to stationary phase in YM broth medium (Difco, Detroit USA), centrifuged and washed in tartrate buffer. Cell suspensions were used to inoculate flasks with tartrate buffer or wine immersed in a thermostatted water bath and stirred magnetically. Samples were collected at regular intervals, diluted and plated in YM agar (Difco, Detroit USA) and incubated at 30ºC for 3 days. D and Z values were calculated using survival cell counts.

Table 1. D values determined in buffer with different pH at 52ºC.

	D. *bruxellensis*	*Dekkera* 093
pH	D_{52}	D_{52}
4,5	1,03	1,26
4,0	0,96	0,97
2,5	0,88	1,20

RESULTS AND DISCUSSION

In studies employing tartrate buffer as the heating menstruum, measurable thermal inactivation began only at temperatures of 50ºC. When heating was performed in wine, significant inactivation begins at 32.5ºC. In the range of pH values tested (2.5 to 5.0), this factor showed no significant influence in the heat inactivation kinetics (Table 1). In the experiments done with different phenolic acids, all compounds caused a decrease in the D values, ferrulic acid having the strongest effect (Table 2). Results from experiments in buffer with added ethanol suggest that the greater heat

Table 2. D values for the inactivation of *Dekkera* strains in buffer with added hydroxycinnamic acids

	D. *bruxellensis*		*Dekkera* 093	
	D_{45}	D_{50}	D_{45}	D_{50}
Control	56,0	3,28	69,7	3,89
Ferulic Acid	2,43	0,24	3,35	-
Caffeic Acid	10,9	1,12	12,3	0,86
Vanilic Acid	13,3	0,57	14,6	1,08
Gallic Acid	12,0	1,22	36,6	1,52

Table 3. D values determined in buffer with 12% (v/v) ethanol added.

	D. *bruxellensis*		*Dekkera* 093	
	$D_{42,5}$	D_{45}	$D_{42,5}$	D_{45}
Control	-	17,4	-	17,6
12% Ethanol	1,33	0,68	1,05	0,25

sensitivity shown in wines can be largely attributed to ethanol (Table 3), although potentiation of this effect might be due to the phenolic content (Figure 1).

Table 4. Estimated values for D and Z for the inactivation of *Dekkera* strains in different mediums.

Medium	Temp. (ºC)	D. *bruxellensis*		D. *anomala*		*Dekkera* 093	
		D_T (min)*	Z (ºC)	D_T (min)*	Z (ºC)	D_T (min)*	Z (ºC)
Tartrate buffer	45,0	56,0	4,8	53,4	4,1	69,7	4,0
	50,0	3,3		2,3		3,9	
	52,5	0,7		0,7		0,7	
Wine A	32,5	23,3	3,5	8,9	3,9	8,6	6,2
	35,0	2,3		1,4		2,6	
	37,5	0,7		0,4		0,8	
Wine B	32,5	14,8	4,6	7,9	4,4	20,2	5,6
	35,0	2,1		4,0		2,2	
	37,5	1,0		0,6		0,9	

* Average values of three determinations
Wine A - 12,8% ethanol, pH 3,51; Wine B – 12,0% ethanol, pH 3,60.

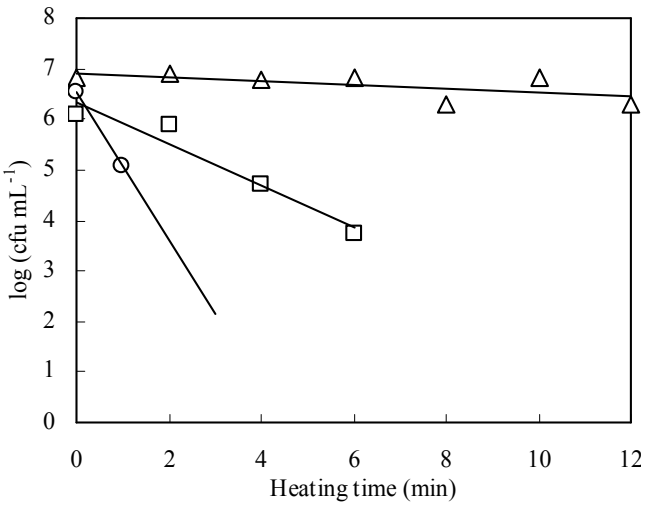

Figure 1. Thermal inactivation of D. *bruxellensis* PYCC 4801 at 45ºC in tartrate buffer (△), buffer with 500 mg L^{-1} ferulic acid (□) and with 12% ethanol (○).

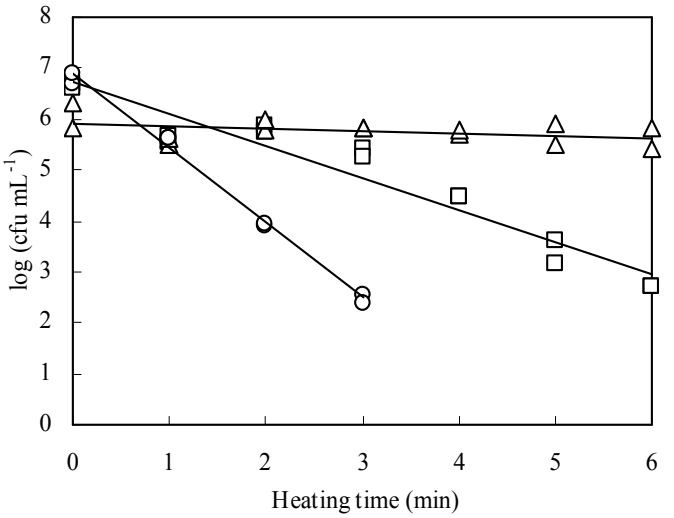

Figure 2. Thermal inactivation of D. *bruxellensis* PYCC 4801 in wine A, at 32,5ºC (△), 35ºC (□) and 37,5ºC (○).

Analysing the results obtained in wine (Table 4), there is a slight difference in the heat resistance of the three strains. Apparently, the strain isolated from wine is more resistant in wine than the collection ones. There is also a difference in heat resistance of the collection strains in wine A and B, which might be explained by the difference in ethanol concentration of the two wines.

Table 5. Heat treatment regimes, determined for the conditions of the tests and in the two wines tested, for a 6D reduction of population.

Wine A						Wine B					
D. *bruxellensis*		D. *anomala*		*Dekkera* 093		D. *bruxellensis*		D. *anomala*		*Dekkera* 093	
Time (min)	Temp. (ºC)	Time (min)	Temp. (ºC)	Time (min)	Temp. (ºC)	Time (min)	Temp. (ºC)	Time (min)	Temp. (ºC)	Time (min)	Temp. (ºC)
4,23	37,50	2,66	37,50	4,80	37,50	6,29	37,50	3,34	37,50	5,68	37,50
0,42	40,95	0,27	41,39	0,48	43,67	0,63	42,10	0,33	41,87	0,57	43,11
0,04	44,40	0,03	45,27	0,05	49,85	0,06	46,71	0,03	46,24	0,06	48,71

As an illustration 6D inactivation regimes are, in wine A and for Dekkera 093, 37.50ºC for 4.80 min or 43.67ºC for 0.63 min (Table 5).

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