Research Journal of Agricultural Science, 45 (4), 2013

THE INFLUENCE OF TEMPERATURE ON THE GROWTH LIMIT OF ACETIC BACTERIA PRESENT IN SAUVIGNON WHITE WINE WITH A CONCENTRATION OF 13% ALCOHOL

Carmen VLĂDULESCU, Gh. MATEI
University of Craiova, No. 13, A.I. Cuza Street

Abstract

Temperature is a determinant factor of the vital-metabolic processes of acetic bacteria. Changing wine into vinegar progresses more rapidly as the temperature is raised. Volatile acid alteration is at the beginning twice faster at 28^0C than at 23^0C and twice faster at 18^0C than at 23^0C. The scale of heating values along which starting from minimum temperature and to the maximum, the bacteria are able to perform normal vital activity, represents the area of growth temperature. Broad limits which extends the range (0 - 45^0C) places acetic bacteria in mesophilic microorganisms category for which the limit temperature increase is 40 - 45^0C. The limit temperature for thermal destruction or death ran for acetic bacteria, between 55 - 60^0C. The optimum temperature for growth for vital processes to take place with maximum intensity, is conventionally defined as the temperature at which cell proliferation is the most intense mode. However, this value does not coincide with that of the thermal optimum temperature for physiological activities of the cell. Maximum temperature of growth supported by acetic bacteria is the greatest heat where the biological activity is still possible, and multiplication of such micro-organisms can still be carried out. Exceeding this threshold heat affects reproductive capacity of acetic bacteria. Minimum temperature for acetic bacteria vary by species between 8 and 10^0C and represents the lowest heat at which cell multiplication, although very slow, can still be achieved. Theoretical the limit of development of vital processes is determined by the freezing temperature of the water. It is important to know, in oenological practice, the optimum growth temperature for the acetic acid bacteria is between 25 - 30^0C. In general, there were no increases in excess of bacteria colony, over 37^0C, but we managed to isolate several thermotolerant strains at 37 - 40^0C. At lower temperatures, acetic bacteria are still active even at 10^0C. As a result, it is difficult to draw a strict temperature range in which these bacteria can grow, the temperatures used during the wine making process seem not to affect the growth of acetic bacteria.

Key words: *acetic bacteria, viable cells, white wine, temperature.*

INTRODUCTION

Maximum temperature of growth supported by acetic bacteria is the greatest heat to the biological activity is still possible, and multiplication of such microorganisms can still be carried out. Exceeding this threshold heat affect reproductive capacity of acetic bacteria.

Further increase above the maximum tolerable temperature, results in bacterial cell killing, reaching the so-called thermal destruction limit. Sterilizing action of heat depends on the physiological state of the cell, the composition of the environment and the type of heat (dry or moist). In general, vegetative cells are more sensitive than spores and cells with normal humidity are also more sensitive than dried - the high degree of hydration favoring thermal coagulation of proteins.

Death of a cell population due to heat abundant can be instantaneous if the temperature is too high, so that the process is carried out gradually, the number of surviving cells can be higher, lower, or zero, depending on temperature, duration of

exposure, the initial number of bacteria, the chemical composition and the physical - chemical environment of the species of bacteria, etc.

The sensitivity of cells to the destructive action of high temperatures are expressed conventionally by the thermal death (the temperature at which in 10 minutes are killed all the cells of a given species), and the thermal death (the length of exposure necessary to kill all the cells at a given temperature).

The action of subminimale temperatures acetic bacteria depends on decisive measure of the rate at which interfere temperature falls below 0C. If it is slow, it occurs the crystallization of water and the electrolyte concentration, resulting in increased osmotic counterpressure in plasmolysis water and electrolyte concentration. If frost is rapidly crystallization tendency of water in the environment will be diminished but it favors the crystallization of intracellular accompanied by electrolyte concentration.

Only in the case of the extremely sudden freezing intracellular crystallization is prevented appearing an amorphous solidification of water in the cell, this leads to a restriction of phenomena with harmful consequences for the cells, but some can therefore survive.

This may explain why organisms support (at least some cells in a population), extremely low temperatures, such as that of boiling liquid hydrogen (-252^0C). On this principle is based "freeze-drying" cultures of bacteria or other germs: apply a sudden cooling (-70^0C) with the aid of dry ice and alcohol, then it is applied the drying by sublimation of water under reduced pressure, changing the cell mass into a dry powder.

Celulele liofilizate se află în stare de anaerobioză, adică sunt fără viaţă dar capabile să trăiască. Repuse în condiţii de mediu favorabile ele îşi reiau procesele vitale exact în formă iniţială.

Lyophilized cells are in anaerobic condition, this means they are lifeless but they are able to live. Restored to favorable environmental conditions they resume their exact original form vital processes.

MATERIAL AND METHOD

The material used for the experiments consisted of:

- White wine 13,0 vol% alcohol, pH = 3, obtained from grapes coming from vineyards Segarcea center. The thermostat was also an indispensable device of our research.

- Viable cells

Analytical methods were related to:

- Determination of sugar in the grapes
- Determination of the alcoholic strength of wine
- Determination of acetic acid in wine
- Determination of sulfur dioxide dosage Microbiological examinations.
- Determination of wine temperature and the environmental temperature.

RESULTS AND DICUSSIONS

Our research on the effect of temperature on growth of acetic bacteria limit in the white wines tried to capture those temperatures which stimulate or inhibit in some way vital processes - metabolic of acetic bacteria present in wine.

We started from a certain microbial load (U.F.C. / ml) and from an acetic acid content of wine, having as oscillating factor the temperature maintained during 30 days while took observations and measurements with thermostat. The results of analyzes are presented in table 1 and table 2 and graphics 1, 2, 3, 4, 5, 6.

When the white wine Sauvignon type has a higher alcohol concentration 13 vol% alcohol, pH = 3 and maintaining the same dose of free SO_2 (active) the sense of the number of processes of cell growth (table 1, table 2 and graphics 3, 4) and increasing acetic acid content of the wine is still preserved.

It is noted, that, due to the higher alcohol concentration of the wine, the rate of increase in the number of cells of acetic bacteria is lower, but acetic acid content of the wine stored at high temperatures over 27^0C is higher.

The above observations confirm that not even the wines with higher concentration of alcohol are not protected against from the vinegar process, if the factors that stimulate metabolic activity of acetic bacteria as temperatures between 17^0C and 40 - 45^0C occur.

Table 1

The influence of temperature on acetic limit growth of bacteria over a period of 30 days, in a white wine (Sauvignon) with an alcohol concentration of 13.0 vol% alcohol, pH = 3.0 and 10 mg / L free SO2

Temperature limits (°C)	Acetic acid content in wine (g/l) H_2SO_4	Initial number of viable bacterial cells UFC/ml	**Determinations after ……days**					
			5		**10**		**15**	
			Acetic acid (g/l) H_2SO_4	The number of viable bacterial cells UFC/ml	Acetic acid (g/l) H_2SO_4	The number of viable bacterial cells UFC/ml	Acetic acid (g/l) H_2SO_4	The number of viable bacterial cells UFC/ml
8-12	0,35	10^2	0,35	10^2	0,35	10^2	0,35	10^2
12-17	0,35	10^2	0,35	10^2	0,35	10^2	0,37	10^2
17-22	0,35	10^2	0,35	10^2	0,35	10^2	0,38	10^2
22-27	0,35	10^2	0,35	10^2	0,35	10^2	0,40	10^2
27-32	0,35	10^2	0,38	10^2	0,46	10^2	0,58	10^3
32-35	0,35	10^2	0,41	10^3	0,47	10^3	0,59	10^4
35-40	0,35	10^2	0,44	10^3	0,50	10^4	0,64	10^4
40-45	0,35	10^2	0,46	10^3	0,58	10^4	0,72	10^4
45-50	0,35	10^2	0,47	10^3	0,66	10^5	0,88	10^5

Table 2

The influence of temperature on acetic limit growth of bacteria over a period of 30 days, in a white wine (Sauvignon) with an alcohol concentration of 13.0 vol% alcohol, pH = 3.0 and 10 mg / L free SO2

Temperature limits (°C)	Acetic acid content in wine (g/l) H_2SO_4	Initial number of viable bacterial cells UFC/ml	**Determinations after ……days**					
			20		**25**		**30**	
			Acetic acid (g/l) H_2SO_4	The number of viable bacterial cells UFC/ml	Acetic acid (g/l) H_2SO_4	The number of viable bacterial cells UFC/ml	Acetic acid (g/l) H_2SO_4	The number of viable bacterial cells UFC/ml
8-12	0,35	10^2	0,35	10^2	0,35	10^2	0,35	10^2
12-17	0,35	10^2	0,37	10^2	0,39	10^3	0,42	10^3
17-22	0,35	10^2	0,40	10^2	0,44	10^3	0,52	10^3
22-27	0,35	10^2	0,46	10^3	0,54	10^3	0,62	10^3
27-32	0,35	10^2	0,66	10^3	0,74	10^4	0,86	10^4
32-35	0,35	10^2	0,74	10^4	0,86	10^5	0,93	10^5
35-40	0,35	10^2	0,82	10^5	0,99	10^6	1,20	10^7
40-45	0,35	10^2	0,89	10^5	1,24	10^6	1,44	10^7
45-50	0,35	10^2	0,98	10^5	1,49	10^6	1,79	10^7

- Determination after 5 days

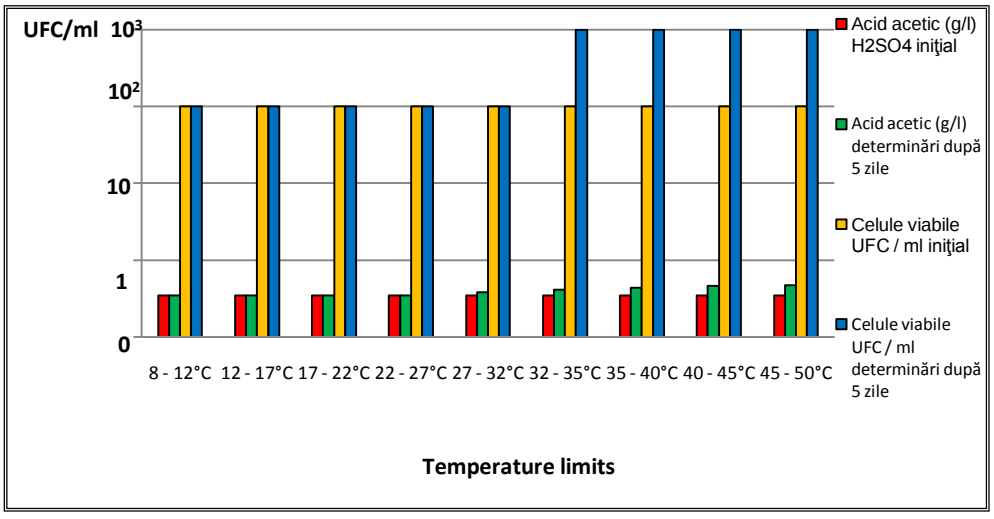

Figure 1. The influence of temperature on acetic limit the growth of bacteria over a period of 30 days, a white wine (Sauvignon) with an alcohol concentration of 13.0 vol% alcohol, pH = 3.0 and 10 mg / L free SO_2

- Determination after 10 days

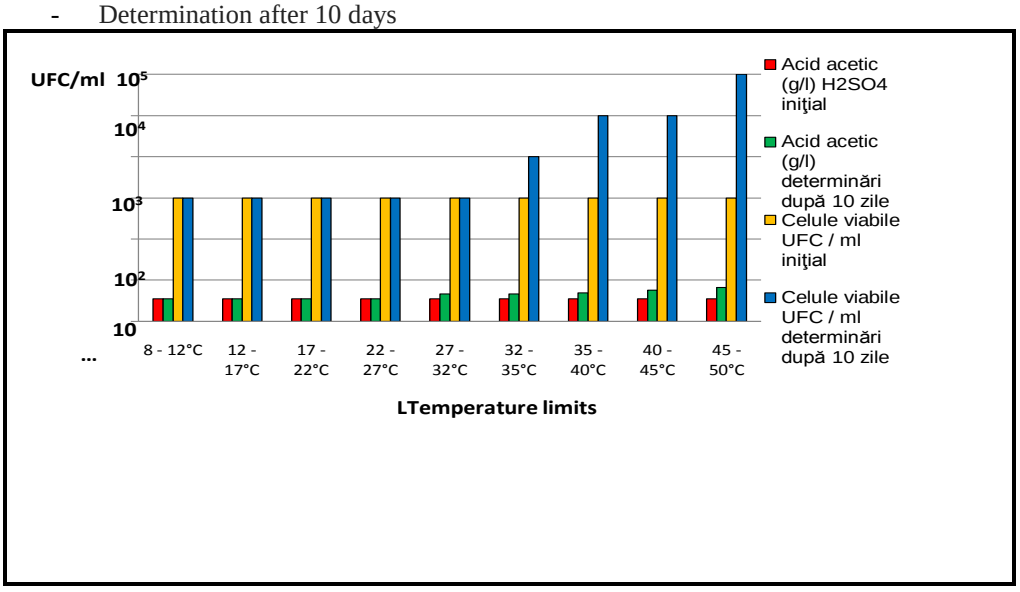

Figure 2. The influence of temperature on acetic limit the growth of bacteria over a period of 30 days, a white wine (Sauvignon) with an alcohol concentration of 13.0 vol% alcohol, pH = 3.0 and 10 mg / L free SO_2

- Determination after 15 days

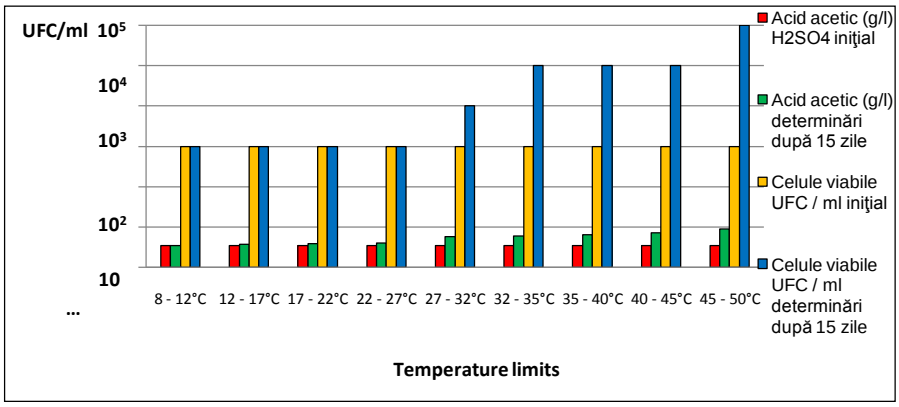

Figure 3. The influence of temperature on acetic limit the growth of bacteria over a period of 30 days, a white wine (Sauvignon) with an alcohol concentration of 13.0 vol% alcohol, pH = 3.0 and 10 mg / L free SO_2

- Determination after 20 days

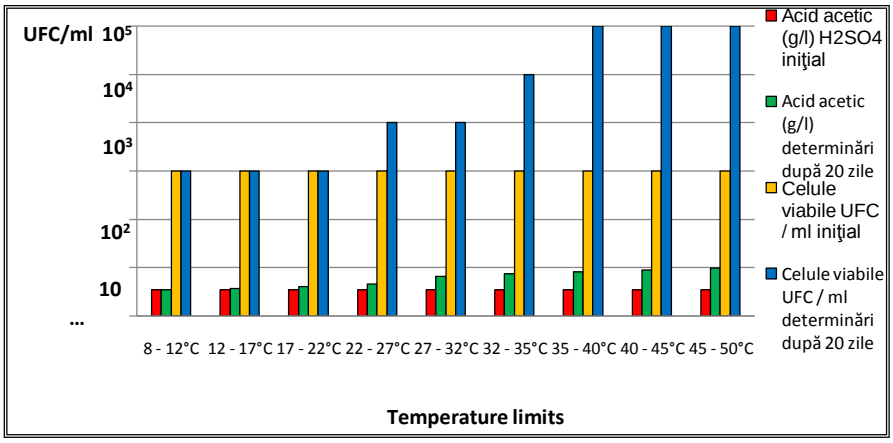

Figure 4. The influence of temperature on acetic limit the growth of bacteria over a period of 30 days, a white wine (Sauvignon) with an alcohol concentration of 13.0 vol% alcohol, pH = 3.0 and 10 mg / L free SO_2

- Determination after 25 days

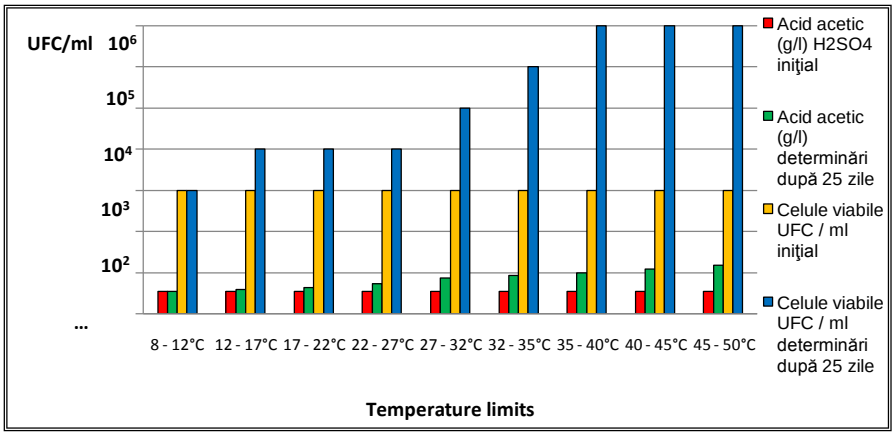

Figure 5. The influence of temperature on acetic limit the growth of bacteria over a period of 30 days, a white wine (Sauvignon) with an alcohol concentration of 13.0 vol% alcohol, pH = 3.0 and 10 mg / L free SO_2

- Determination after 30 days

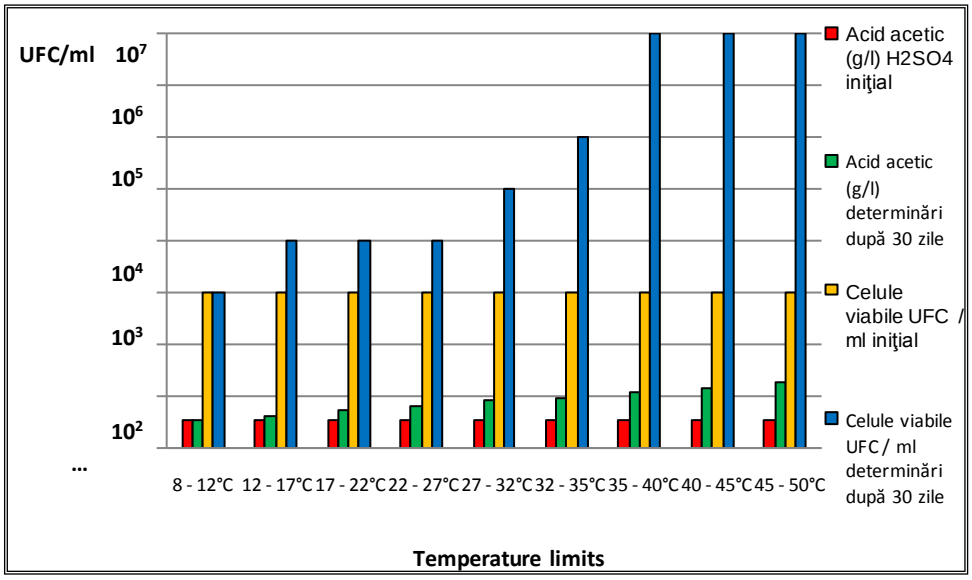

Figure 6. The influence of temperature on acetic limit the growth of bacteria over a period of 30 days, a white wine (Sauvignon) with an alcohol concentration of 13.0 vol% alcohol, pH = 3.0 and 10 mg / L free SO_2

CONCLUSIONS

Temperature is a determinant factor; vinegar process progresses faster as the temperature is higher, volatile acidity formed in the early alteration is two times higher at 28^0C than at 23^0C and twice faster than at 18^0C than at 23^0C.

It is important to know, in oenological practice, the optimum growth temperature for the acetic acid bacteria is in the range of 25 - 35^0C.

At lower temperatures acetic bacteria are still active and 10^0C as well. Therefore it is difficult to draw a strict temperature range in which these bacteria can grow, temperatures used during the wine making process seem not to affect the growth of acetic bacteria.

The acetic growth limit of bacteria is influenced by the temperature. Temperatures of 8 - 12^0C maintained during storage of white wines regardless of their alcohol concentration may maintain vital processes in place - Metabolism of acetic bacteria.

Temperatures above 12^0C and up to 45 - 50^0C stimulate the multiplication of acetic bacteria and increase the metabolic processes by which wines are rich in acetic acid content making them unacceptable for consumption.

In all cases, regardless of alcoholic white wines, maintained at 60 - 65^0C for 2 to 10 minutes acetic bacteria cells are inactivated.

BIBLIOGRAPHY

1.BULTON C. A. - Physiological conditions of yeast and achievements in fermentation. Bioscience nr. 4, 1998.

2.GHEORGHITA M, MUNTEAN CAMELIA, BADUCA C. - Oenologie, vol. 2. Ed. Sitech, Craiova, 2002.

3.JOYEUX A., LAFON-LAFOURCADE S., RIBEREU-GAYON P. - Evolution of acetic acid bacteria during fermentation and storage of wine. *Appl Environ Microbiol* 48, 1984.

4.MILLET V., LONVAUD-FUNEL A. - The viable but non-culturable state of wine microorganisms during storage. *Lett Appl Microbiol* 30, 2000.

5.POPA A. - Secretul vinului bun. Ed. Alma Craiova, 2008.

6.RADU I. F., GHERGHI A. - Păstrarea şi prelucrarea produselor hortivinicole. Ed. Agro-Silvică, Bucureşti, 1967.

7.TEODORESCU ŞT., POPA A., SANDU G. - Oenoclimatul României. Vinurile României şi climatul lor caracteristic, Editura Ştiinţifică şi Enciclopedică, Bucureşti, 1987.

8.VLADULESCU CARMEN - Teza de doctorat, Universitatea din Craiova, 2010.