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ORIGINAL RESEARCH PAPER

THE INFLUENCE OF CULTURE MEDIA ON ACETIC FERMENTATION WITH SELECTED *Acetobacter* STRAINS

Maria Cristiana Garnai

"Dunarea de Jos" University of Galaţi, Food Science and Engineering Faculty, Department of Biochemical Technologies, 47 Domneasca Street, 800201, Galaţi, Romania

Corresponding author: maria.garnai@ugal.ro, cristianamt@yahoo.com

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Abstract: We have systematically followed the efficiency of acetic fermentation, by cultivating 14 *Acetobacter* strains (previously isolated and identified), within a medium obtain out of ethanol and acetic acid, in various proportions, and utilizing corn extract (CE) as a nutrient.
The purpose of the research was to determine the resistance of the studied *Acetobacter* strains related to the composition of the cultivation media (acidity and alcohol content of the medium), as well as following the dynamics of the acetic fermentation by calculating the practical yield.
The research led to optimal variants which may be industrially exploited in order to obtain vinegar.

Keywords: *acetic bacteria, acetic fermentation, acidified hydro-alcoholic medium*

INTRODUCTION

A modern vinegar production technology supposes the use of selected acetic bacteria during fermentation [1]. For this purpose we have studied pure cultures of acetic bacteria, in order to discover their fermentative potential, with different variants of the raw materials and of the composition of the fermentation medium.
14 *Acetobacter* strains (previously isolated and identified) have constituted the biological material of our research. Out all of them, the acetic bacteria strains noted T5, T6 and T8 presented a conversion yield of alcohol into acetic acid below 50%, within all media variants under study; that is why they were never been used subsequently. The T14 acetic bacterium strain (*Acetobacter aceti xilinum*) was not analyzed during this research because it does not have any industrial importance (as a fermentation agent). In conclusion, the research was conducted over the rest of ten acetic bacteria strains.
These cultures were obtained after isolation from alcoholic media (wine and cider). The cultivation of these bacteria was done in identical conditions regarding fermentation media (alcohol, acetic acid and corn extract) and thermostatic conditions (25°C). The samples of fermentation media were introduced into fermentation flasks and inoculated each with 5% *Acetobacter* culture (taken into study). As a nutrient we have used the indigenous corn extract in 1% ratio.

MATERIALS AND METHODS

Materials:

- the acetic bacteria species isolated and identified prior to this study;
- ethylic alcohol (96% v/v);
- glacial acetic acid;
- corn extract (byproduct of Starch and Glucose Factory of Târgu Secuiesc, strongly appreciated in the field literature as rich in nutrients (especially, as a cheap source of nitrogen).

Methods:

- determination of total acidity by titrimetric method (g acetic acid/100 mL sample);
- determination of ethanol by ebuliometric method (with Dujardin-Salleron alcoholmeter) (% vol);
- determination of etanol by picnometric method.

Methodology:

In a static system, in glass flasks and at a temperature of 25°C, in samples of 200 mL, in identical conditions, we cultivated ten *Acetobacter* strains; one of them industrial (noted Ai), and nine strains isolated from wines and cider (noted A10, T10, 2Gl, 3Gl, S, Bz, 3Gi, C2, C3) coming from three counties: Dâmbovița, Galați and Buzău.
The five cultivation media (V1 – V5) had in common the nutrient in use (1% corn extract). The acetic acid content of the fermentation media was varied between 2 and 6%, while alcohol was varied between 9 and 5%. In laboratory conditions, seeding

media with acetic bacteria was done, with all samples, in identical conditions, with the same quantity of inoculum (5%). Table 1 shows the composition of the cultivation media.

Table 1. *Cultivation media composition function of medium variants*

Medium variants	Culture medium composition (% vol)		
	Acetic acid	Ethanol	Corn extract
V_1	2	9	1
V_2	3	8	1
V_3	4	7	1
V_4	5	6	1
V_5	6	5	1

RESULTS AND DISCUSSION

The research undertaken with ten *Acetobacter* strains, in five cultivation medium variants, reveals the sensibility and the resistance of each strain to the initial acidity of the cultivation medium. For each of the experimented acetic bacteria strain, we were able to underline the most important aspects regarding the optimal variants of the cultivation media, from point of view of the efficiency of the fermentative activity.
It is well known that, in the industrial process of obtaining vinegar, from batch to batch, a certain quantity of vinegar is kept in the fermentor in order to insure, on one hand, the acidity necessary to the fermentation medium to prevent infections and, on the other hand, in order to seed the fermenting leaven with acetic bacteria [2 – 5].
In the process of establishing these conditions, one must take into account the properties and the behavior of each *Acetobacter* used as a fermentation agent.
The acetic fermentation was observed during a period of 42 days; we have periodically determined acidity and, at the end, the residual ethylic alcohol.
For the purpose of our research, we further on analyzed the results regarding:
- the resistance of the studied *Acetobacter* strains to the cultivation media composition (acidity and alcohol content of the medium);
- the dynamics of the acetic fermentation through practical yield.
We present, as graphics, the dynamics of the acidity of each *Acetobacter* strain, function of the cultivation medium.
The acidity of the vinegar obtained in medium variant (V1) was between 7.64 – 8.95 g acetic acid/100 mL sample, special note for some of the ten strains under study: T10, 2Gl, A10, S, Ai and C3 (Figure 1). The highest value of the acidity of the vinegar was obtained with T10 strain.
The obtained results demonstrate that the initial acidity of the cultivation medium (2%) favorites the optimal growth of the bacteria cultures under study.
In this medium variant, the quantity of alcohol (9%) was not an impediment to the growth of the acetic bacteria cultures under study.
In the medium variant (V2), the acidity of the vinegar obtained was between 4.88 – 9.18 g acetic acid/100 mL sample (Figure 2), special note for eight out of the ten strains under study: T10, C3, C2, 2 Gl, 3 Gl, A10, Ai and S. The highest value of the acidity of the vinegar was obtained with T10 strain.

The results we obtained (except for strains Bz and 3Gi) demonstrate that the initial acidity of the cultivation medium (3%) is benefic for the optimal growth of the bacteria cultures under study.
In this medium variant, the quantity of alcohol (8%) was not an impediment to the growth of the acetic bacteria cultures under study.

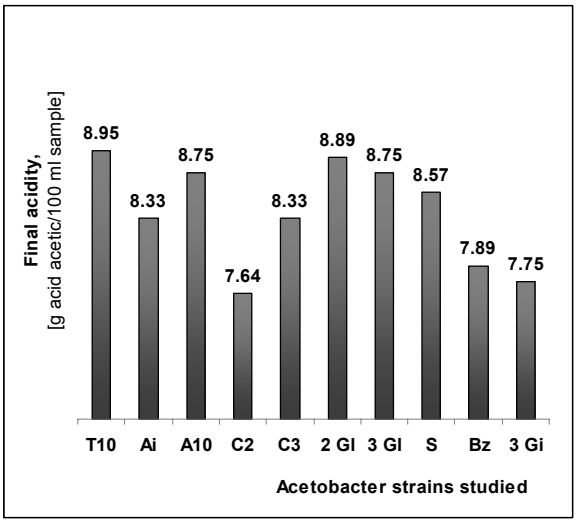

Figure 1. *Final acidity of the Acetobacter strains in cultivation medium V1*

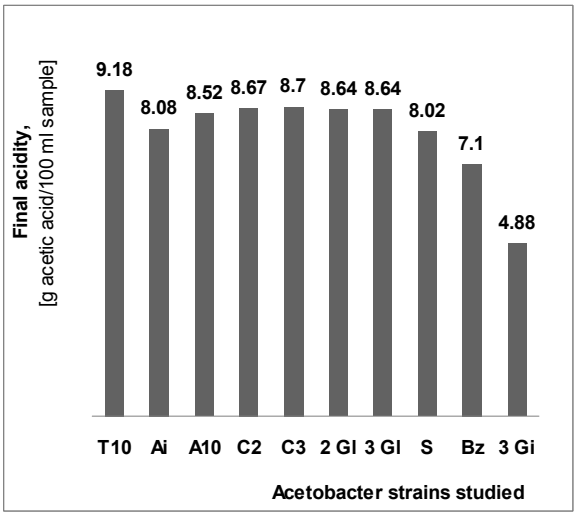

Figure 2. *Final acidity of the Acetobacter strains in cultivation medium V2*

The acidity of the vinegar obtained in medium variant V3 (Figure 3) was between 4.50 – 9.39 g acetic acid/100 mL sample, special note for two out of the ten strains under study: T10 and C2, but we have also got good results with strains A10, 2 Gl, C3, Ai, 3 Gl and S.
The highest value of the acidity of the vinegar was also obtained with T10 strain.
The results we obtained (except for strains Bz and 3Gi) demonstrate that the initial acidity of the cultivation medium (4%) favors the growth of the bacteria cultures under study.
In this medium variant, the quantity of alcohol (7%) was not an impediment to the growth of the acetic bacteria cultures under study.

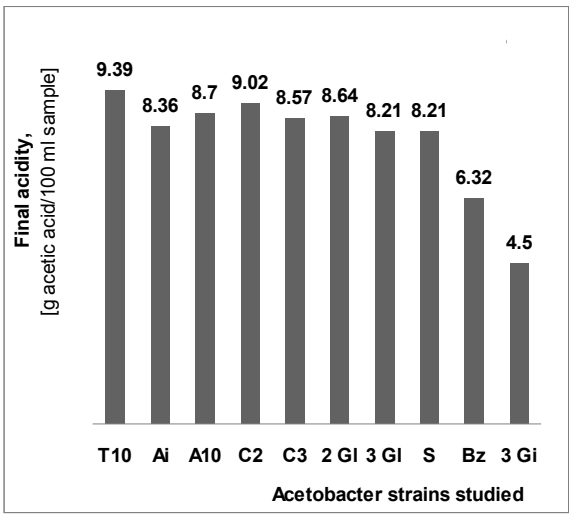

Figure 3. *Final acidity of the Acetobacter strains in cultivation medium V3*

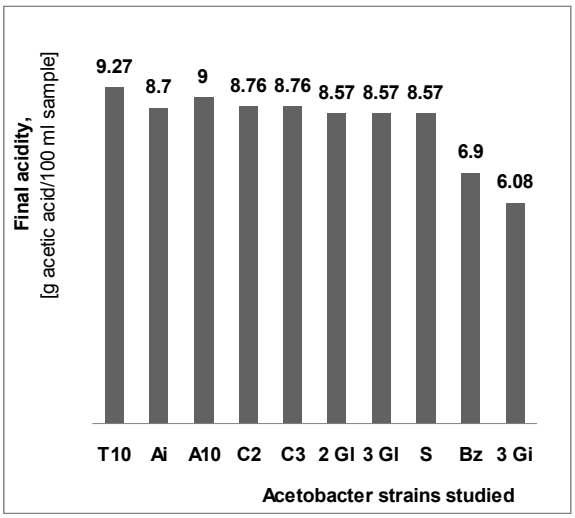

Figure 4. *Final acidity of the Acetobacter strains in cultivation medium V4*

The acidity of the vinegar obtained in medium variant V4 was between 6.08 – 9.27 g acetic acid/100 mL sample, special note for five out of the ten strains under study: T10, A10, C3, C2 and Ai. We have also got good results with strains 2Gl, 3Gl and S (Figure 4).

The highest value of the acidity of the vinegar was obtained with T10 strain.

The results we obtained (except for strains Bz and 3Gi) demonstrate that the initial acidity of the cultivation medium (5%) favors the optimal growth of the bacteria cultures under study.

In this medium variant, the quantity of alcohol (6%), correlated with high acidity, was not an impediment to the growth of the acetic bacteria cultures under study.

The acidity of the vinegar obtained in medium variant V5 (Figure 5) was between 6.63 – 8.90 g acetic acid/100 mL sample, special note for seven out of the ten strains under study: C3, Ai, 3 Gl, S, A10, 2Gl and T10. The highest value of the acidity of the vinegar

was obtained with C3 strain, taking special note that strain T10 was significantly affected by the medium composition.

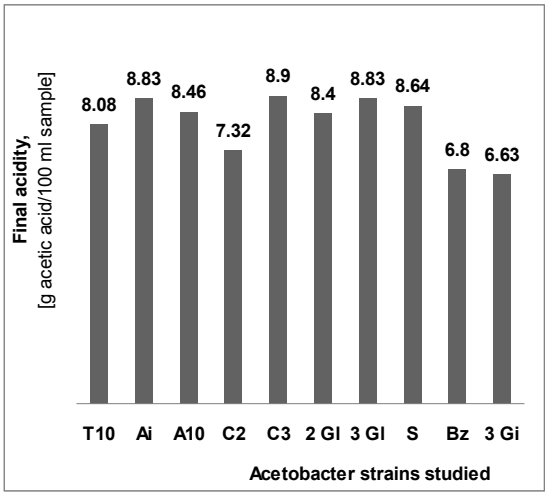

Figure 5. *Final acidity of the Acetobacter strains in cultivation medium V5*

The results we obtained demonstrate that the initial acidity of the cultivation medium (6%) affected the optimal growth of the bacteria cultures under study, all of the strains presenting reduced acidity (compared to the previous working variants).
The quantity of alcohol of the medium (5%), correlated with high acidity (6%), represented an impediment in the growth of the acetic bacteria cultures under study.
In the following tables are presented the practical yields attained by the *Acetobacter* strains function of the culture medium (V1 – V5).
The practical yield [%] was calculated according to the formula:

$$\eta_p = \frac{final\ acidity - initial\ acidity}{initial\ alcohol - final\ alcohol} \cdot 100$$

Within the culture medium with **2% acetic acid and 9% alcohol** (spirit) (**V1**), all acetic bacteria strains realized practical yields of over 60%, special note for the strains T10, 2 Gl, 3 Gl, A10, C3 and S. Strain T10 had the best yield, i.e. 78%.
Within the medium with **3% acetic acid and 8% alcohol (V2)**, eight of the acetic bacteria strains taken initially under study, we realized yields of over 62%, special note for the strains C3, A10, 3Gl, 2 Gl, C2, S, Ai. Maximum yield of 76% was reached by the *Acetobacter* strain T10.
Cultivation of the acetic bacteria within a medium with **4% acetic acid and 7% alcohol (V3)**, pH more acid, influenced the growth of two of the strains (Bz, 3Gi), which proved to be more sensitive. Eight of the strains under study have obtained yields over 61%. Maximum practical yield of 76% was realized by the T10 strain, and the C2 strain realized 75%.
Within the fermenting medium containing **5% acetic acid and 6% alcohol (V4)**, we realized practical yields of over 63% with 7 strains. The maximum practical yield of 83% was obtained with the C2 strain, followed by 3Gl with 80% and by T10 with 75%. Note: T10 strain was slightly affected by the initial acidity of the medium.

Table 1. *Practical yield realized by the Acetobacter strains function of the culture medium (V1-V5)*

Culture medium	Composition of the culture medium	Strain of *Acetobacter* innoculated [5%]	Practical yield [%]
V1	2% acetic acid 9% ethanol 1% corn extract	T10	**78**
		Ai	67
		A10	*72*
		C2	60
		C3	70
		2 Gl	***73***
		3 Gl	***73***
		S	70
		Bz	61
		3 Gi	62
V2	3% acetic acid 8% ethanol 1% corn extract	T10	**76**
		Ai	62
		A10	*69*
		C2	65
		C3	***71***
		2 Gl	66
		3 Gl	*69*
		S	65
		Bz	48
		3 Gi	26
V3	4% acetic acid 7% ethanol 1% corn extract	T10	**76**
		Ai	61
		A10	65
		C2	***75***
		C3	65
		2 Gl	*67*
		3 Gl	63
		S	63
		Bz	39
		3 Gi	9
V4	5% acetic acid 6% ethanol 1% corn extract	T10	*75*
		Ai	66
		A10	71
		C2	**83**
		C3	70
		2 Gl	58
		3 Gl	***80***
		S	63
		Bz	43
		3 Gi	27
V5	6% acetic acid 5% spirit alcohol 1% corn extract	T10	*75*
		Ai	66
		A10	71
		C2	**83**
		C3	70
		2 Gl	58
		3 Gl	***80***
		S	63
		Bz	43
		3 Gi	27

The medium with **6% acetic acid and 5% alcohol (V5)** proved to be less favorable for the ten strains of *Acetobacter* under study. The least affected by initial acidity were strains Ai, 3 Gl and A10, which realized a practical yield of over 70%. The best yields were realized by strains Ai (74%) and 3 Gl (73%).
Subsequently to the analysis of the practical yields, the best strains of acetic bacteria function of the culture media are shown in Table 2.

Table 2. *Selecion of acetic bacteria strains function of yield and culture media*

Culture media				
V1	**V2**	**V3**	**V4**	**V5**
T10 (78%)	**T10** (76%)	**T10** (76%)	**C2** (83%)	**Ai** (74%)
2 Gl, 3 Gl (73%)	**C3** (71%)	**C2** (75%)	**3Gl** (80%)	**3 Gl** (73%)
A10 (72%)	**A10** (69%)	**2 Gl** (67%)	**T10** (75%)	**A10** (70%)

CONCLUSIONS

The study has underlined the optimal variants, as a function of the *Acetobacter* strain used, and of the fermenting medium composition. The best practical yield strains in this study were T10, C2 and 3Gl.
T10 *Acetobacter* strain showed an increased resistance to the initial acidity of the cultivation media, with 2, 3, 4 and 5% acetic acid (V1 – V4), without any effects over the multiplication process and cell activity, which assure a strong vinegar of 9 – 9.39% acetic acid, and with a maximum favorable yield of 78%, within the cultivation media of 2, 3 and 4% acetic acid.
C2 *Acetobacter* strain showed an increased resistance to the initial acidity of the cultivation media, with 4 and 5% acetic acid (V3 – V4), when we obtained a vinegar of 8.76 – 9.02% acetic acid and a favorable practical yield of 75 - 83%.
3Gl *Acetobacter* strain showed an increased resistance to the initial acidity of the cultivation media, with 2, 5 and 6 % acetic acid (V1, V4 – V5), but has given a strong vinegar of 8.57 – 8.83% acetic acid, and a conversion practical yield of 73 - 80%.

The fermentative qualities of **T10, C2 and 3 Gl** ***Acetobacter*** strains ***highly recommend them as fermentation agents*** in the ***technology of obtaining vinegar out of fermentation alcohol***, with the use of corn extract as a nutriment, a sub-product of food industry.

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