Monitoring the Diversity of the Yeasts Population which are Present in the Musts Obtained from the Grapes Harvested in Iordana-Apold, Riesling Italian –Blaj, Royal Feteasca Jidvei and Hárslevelű – Tokaj

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Abstract
The paper's aim is to emphasize the variety of the yeasts populations which are present in the musts from inland and Tokaj areas. The study is based on the alcoholic fermentation on the four strains which were selected for this purpose, the isolation of the specific yeasts and the quantification of the obtained results through in the data base. From the fermentative dynamics' point of view, the differences occur depending on the growing environment and on the strain types which were considered.The minerals play an important role in increasing the biotechnological properties of the wine yeasts *Saccharomyces bayanus*, *Saccharomyces ellipsoideus* regarding the fermentative activity and multiplication speed.

Keywords: alcoholic fermentation, grapes species, yeasts.

1. Introduction

The alcoholic fermentation is a complex process through which the grapes must, under the action of the microorganisms and especially of the yeasts, is transformed into wine [1-3]. The organoleptic properties of the final wine depend on the type of yeasts which is predominant and on its properties. The predominant yeasts from this fermentation are *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. The bio-diversity is completed with other yeasts like *Saccharomyces pombe*, *Pichia*, *Torulaspora* etc. Depending on the area from which the grapes are gathered, every species of yeast provides to the wine a specific locality and individual flavours and special attributes [4, 5].
The present study aims to isolate yeast strains from four different areas, to characterize them regarding their fermentative power, and finally to form a base which to predict their usability.

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2. Materials and methods

We selected four monitoring areas with the following grape variety: Iordana-Apold, Italian Riesling–Blaj, Royal Feteasca Jidvei and Hárslevelű – Tokaj. From these areas we harvested grapes from which we obtained must which was the base of the wine yeasts. The musts were subject to alcoholic fermentation at 22ºC for 10 days. We sampled three different stages of the alcoholic fermentation (after the first day, in the tumultuous phase and at the end of alcoholic fermentation), and each time we isolated the yeasts which were specific to that time from the growing environments yeast-peptona – glucose-agar. The isolated samples were phenotypicly identified and were subject to specific tests [5-7].
Later, we selected 4 yeast strains of *Saccharomyces ellipsoideus* named SEIA, SERI,

SEFR and SEHT, and 4 yeast strains of *Saccharomyces bayanus* named SBIA, SBRI, SBFR and SBHT which were subject to alcoholic fermentation in fermenter Startorius equiped with sensors for: temperature, biomass, CO_2, dissolved oxygen**,** oxygen released, monitoring their behaviour in three different growing environments [8]:
- must malt (M1),
- must malt, ($MgSO_4$) x $7H_2O$ 0,5 g/l (M2),
- must malt, $(NH_4)_2SO_4$ 1 g/l (M3).

3. Results and discussion

The sampling was for the beginning of the fermentation (I), then for the tumultuous phase (T) and for its end (F). The yeasts were isolated using the impoverishment of the loop resulting the configuration presented in the following table:

Table 1. The number of isolated colonies from the following grapes: Iordana, Italian Riesling, Royal Feteasca and Hárslevelű

Yeast strains	Iordana-Apold grapes			Italian Riesling grapes-Blaj			Royal Feteasca grapes-Jidvei			Hárslevelű grapes-Tokaj		
	I	T	F	I	T	F	I	T	F	I	T	F
Saccharomyces pombe	2	3	1	1	2	1	2	1	2	3	4	1
Saccharomyces bayanus.	0	3	6	1	5	3	2	4	5	0	1	2
Saccharomyces ellipsoideus	11	23	16	12	25	17	12	34	24	11	25	15
Torulopsis bacillaris	0	1	3	2	1	0	1	3	1	1	2	2
Mycoderma vini	0	1	0	0	0	0	0	1	0	0	1	1
Pichia kluyveri	0	2	0	0	0	0	0	2	0	0	0	0

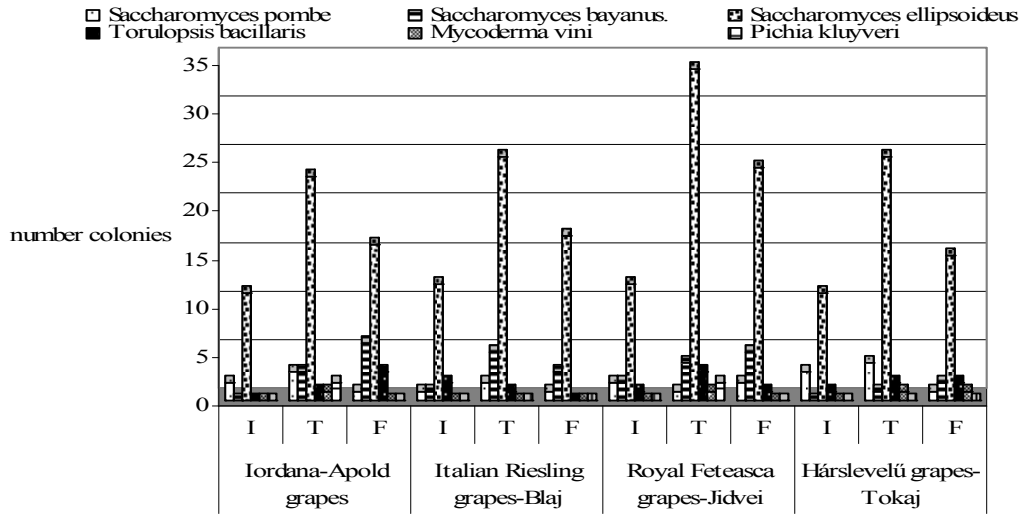

Figure 1. The relationships between the isolated colonies from the following types of grapes: Iordana, Italian Riesling, Royal Feteasca and Hárslevelű

As it can be observed from the Figure 1, we can say that during the tumultuous phase we find more *Saccharomyces ellipsoideus*, followed by *Saccharomyces bayanus* yeasts. Watching the fermentative activity of the four yeast strains, *Saccharomyces ellipsoideus* in the three growing environments, we observed that the environment influences the fermentative process due to the chemicals it contains. From the fermentative dynamics' point of view, the differences occur depending on the growing environment and on the strain types which were considered.
The obtained results are presented in figure 2 from where we can conclude that the tumultuous phase is observable from the day 4 for the SEIA strain in M3 growing environment, followed by SERI and

SEFR strains in M1 growing environment and SEHT strain in the M3 environment starting withe the day 5.

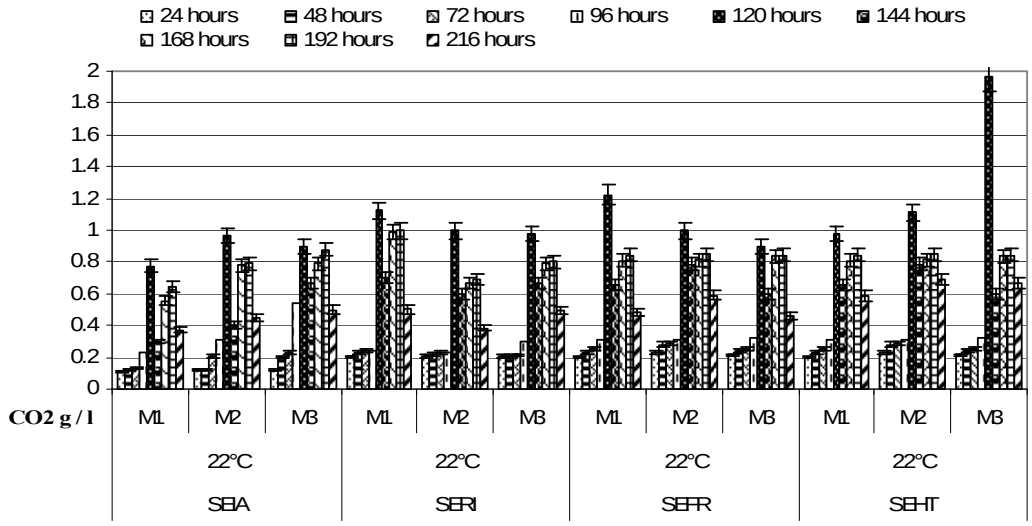

Figure 2. The evolution of the fermentative activity of the *Saccharomyces ellipsoideus* yeast strains SEIA, SERI, SEFR, SEHT in M1, M2 and M3 growing environments

After the 5th day of fermentation we can observe significant oscillations for all four strains in the M1, M2 and M3 growing environments. The most significant differences can be observed for the SEHT strain which has best yield in the fifth day in M3 environment with an about 70% more than the other three strains, and this is valid for the entire fermentation stage (best numerical and procentual values). The next days show constant and close values. The SERI and SEFR show close values in M2 and M3, and for M1 they present higher values. The SEIA strain has the best tield in M2 and M3 growing environments compared to M1 and the lowest yield compared to the SERI, SEFR and SEHT strains.

Following this property (the fermentation capacity of the wine yeasts *Saccharomyces ellipsoideus* in the M1, M2 and M3 growing environments) we can say that the SEHT strain has the best fermentative qualities and it is followed by the SEFR, SERI and SEIA strains.

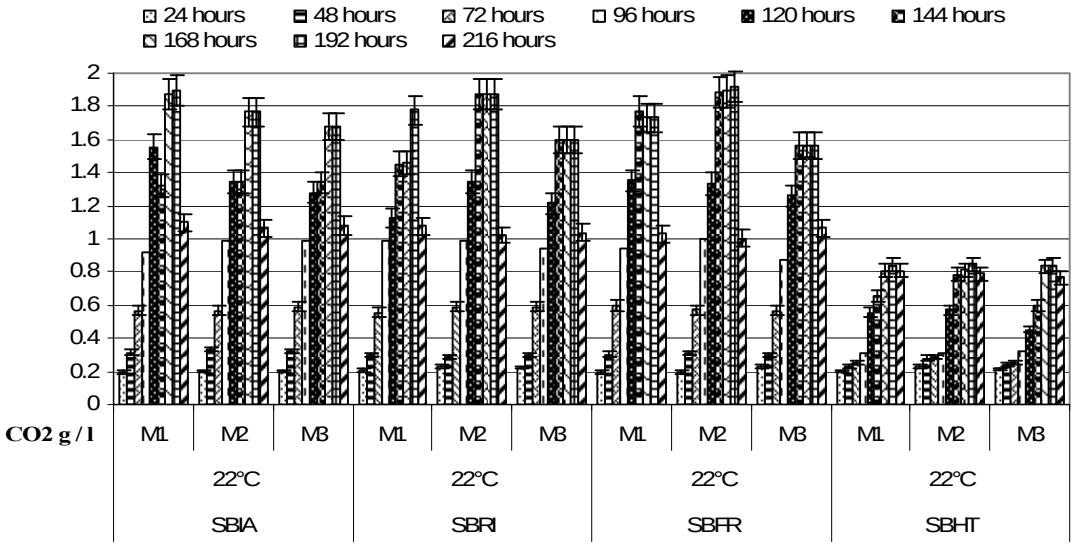

Figure 3. The evolution of the fermentative activity of the *Saccharomyces bayanus* yeast strains SBIA, SBRI, SBFR and SBHT in M1, M2 and M3 growing environments

From the obtained results, the dynamics of the fermentative capacity of the four yeast strains of type *Saccharomyces bayanus,* SBIA, SBRI, SBFR and SBHT, according to the figure 4 we can observe that the tumultuous fermentation is visible only from the 7th day for all the four strains which were subject to this study in the M1, M2 and M3 growing environments, followed by a slight decrease.

The SBIA strain has a very good evolution, resulting the biggest quantity of released CO_2 in the M1 environment. The SBRI and SBFR strains present close values for the M2 and M3 environments, and for M1 lower values.

The SEIA strain has the best yield in M2 and M3 environments compared to M1 and the lowest yield compared to the SERI, SEFR and SEHT strains.

Watching the evolution of the fermentative capacity of the wine yeast strains *Saccharomyces bayanus* in M1, M2 and M3 growing environments we can say that the SBHT strain present the lowest fermentative properties during the entire fermentation phase, due to the low nitrogen which decreases the fermentation speed.

4. Conclusions

We can conclude that the fermentative process is conditioned by the chemical composition of the growing environment for the same yeast strain type.

The minerals play an important role in increasing the biotechnological properties of the wine yeasts *Saccharomyces bayanus*, *Saccharomyces ellipsoideus* regarding the fermentative activity and multiplication speed.

The minerals (magnesium sulphate) play a significant role for obtaining biotechnological products with superior properties.

The relative studies regarding the fermentative activity of the yeast strains *Saccharomyces ellipsoideus and Saccharomyces bayanus* emphasized increased values for the SEHT and SBIA strains.

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