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ASPECTS OF THE INTERSPECIFIC RELATIONSHIPS BETWEEN KLOECKERA APICULATA AND SACCHAROMYCES CEREVISIAE var. ELLIPSOIDEUS YEASTS

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Abstract:

In this paper, we have studied during alcoholic fermentation, some aspects of the interspecific relationships between the apiculated yeasts belonging to the Kloeckera apiculata species and the elliptical yeasts belonging to Saccharomyces ellipsoideus species, isolated from Murfatlar vineyard, viticol centre Cernavoda. The two species yeasts were inoculated separately and mixedly, in sterile Pinot Gris must. At the mixted inoculation, an inhibition have been noticed, particulary for Saccharomyces ellipsoideus species. The inhibition was maximum, when the inoculation with Saccharomyces ellipsoideus species, is done when there is a maximum activity period of Kloeckera apiculata species. Mixed inoculation leads to the occuarence of two maximum in the fermentation process: the first maximum occurs due to the activity of the Kloeckera apiculata species and the second maximum occurs due to the activity of the Saccharomyces ellipsoideus species, after the passing of the inhibition period.

Key words: alcoholic fermentation; inhibition period; mixted inoculation.

INTRODUCTION

In the wine microbiology majority of the researches are orientated upon the relationships between microorganisme species and from inside of the same species. All the time were studied the yeasts attend in the fermentation process [Beleniuc G., 2006; Castelli T.,1973; Gandini -1966], the yeasts influence upon lactique bacterias and viceversa [Ribereau-Gayon and Peynaud-1960, 1961] the action of the acetic bacterias and moulds upon the yeasts and lactic bacterias [Ribereau-Gayon, 2000]. Were studied the relationships between
the mains yeasts groups, apiculate and elliptical [Domerq-1956]. Some authors, have seen the negative role of the apiculate yeasts in alcoholic fermentation process and therefore even recommend their elimination from the must [CoteaD.V., 1985]. The others authors, showed the main role of apiculate yeasts in the wine flavours formation. They showed that the famous wines, can not be obtained in exclusivity with elliptical yeasts, only by using the spontaneous microflora from the vineyard.

MATERIAL AND METHOD

Were studied some aspects of the relationships between apiculate yeasts, belonging to Kloeckera apiculata species and elliptical yeasts, belonging to Saccharomyces ellipsoideus, isolated in the viticol Centre Medgidia, from Murfatlar vineyard, and identified by „Yeasts A taxonomic study, 6-th Revised and Enlarged Edition"[Kurtzman, C.P., şi J.W. Fell, 2006]. The researches were made, using like fermentation medium, Pinot gris sterile must with the following characteristics: 230 g/l sugars and 6,24 g/l H_2SO_4 total acidity.
The experiments were made in seven variants, as following:
V_1 – inoculated only with Kloeckera apiculata species;
V_2 – inoculated only with Saccharomyces ellipsoideus species;
V_3 – inoculated simultaneous with Kloeckera apiculata and Saccharomyces ellipsoideus species;

V_4 – inoculated with Kloeckera apiculata species and after one day with Saccharomyces ellipsoideus species;
V_5 – inoculated with Kloeckera apiculata species and after 2 days with Saccharomyces ellipsoideus species;
V_6 – inoculated with Kloeckera apiculata species and after 4 days with Saccharomyces ellipsoideus species;
V_7 – inoculated with Kloeckera apiculata species and after 31 days with Saccharomyces ellipsoideus species. The samples were keep at 25^0C temperature and was followed the fermentation process by daily yeasts population evolution (daily counting–Thoma mount) and the lost in weight registration, by CO_2 lost (g%).
Finally, the wines obtained were analised from the chemical characteristics point of view, using the O.I.V methods and the Romanian standards in force.

RESULTS AND DISCUTIONS

The results are showed in the charts 1-7 and in the table 1.
Following the curves of the cells yeasts evolution and the lost in weight, for the sample inoculated with one yeasts species (V_1 and V_2), we can show (chart 1): Kloeckera apiculata yeasts has a big fermentative intensity in the first four-five days, and Saccharomyces ellipsoideus species in the first eight days. After these periods, the fermentation has a low intensity. In the power fermentation period (1-5, 1-8 days) the number of yeasts cells are increase and, after this period, was hardly to establish with accuracy a correlation between total yeasts cells number/mm^3 and the fermentation intensity.

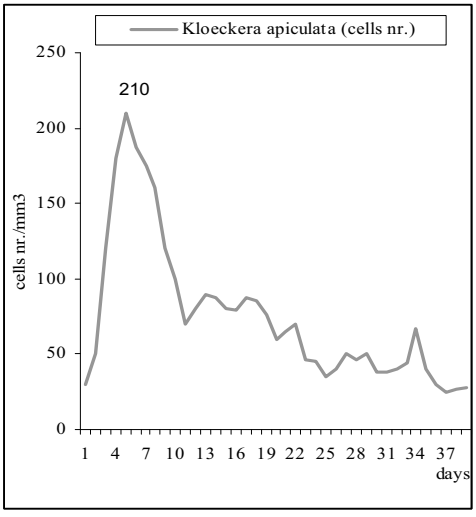

Chart 1. The cells number evolution at V1 (inoculated with Kloeckera apiculata)

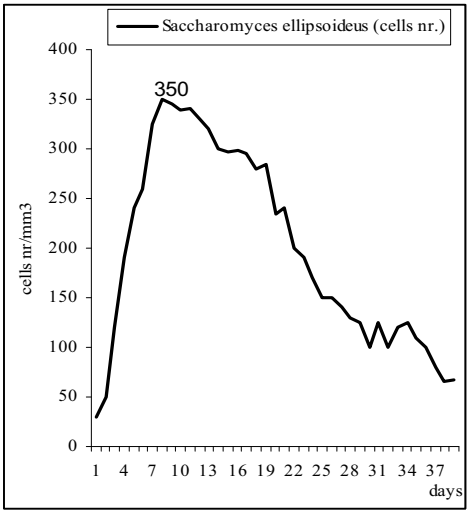

Chart 2. The cells number evolution at V2 (inoculated with Saccharomyces ellipsoideus)

When the samples were mixed inoculated (V_3-V_8), we have seen a mutual inhibition of two yeasts species, correlate with their inoculated moment. When the two yeasts species are simultaneous inoculated (V3, chart 3) was showed a mutual inhibition thus that, the Kloeckera apiculata species can not reach a big cells number like in V_1 (inoculated only with Kloeckera apiculata species). To the other mixed variants inoculated (V_4-V_7) due the lag of inoculation with Saccharomyces ellipsoideus, the yeasts Kloeckera apiculata, achieve a number of cells/mm^3, approached V_1 (inoculated only with Kloeckera apiculata species). Only at the V_7 variant the Kloeckera number of cells/ mm^3, is the same as in V_1 (chart 7).

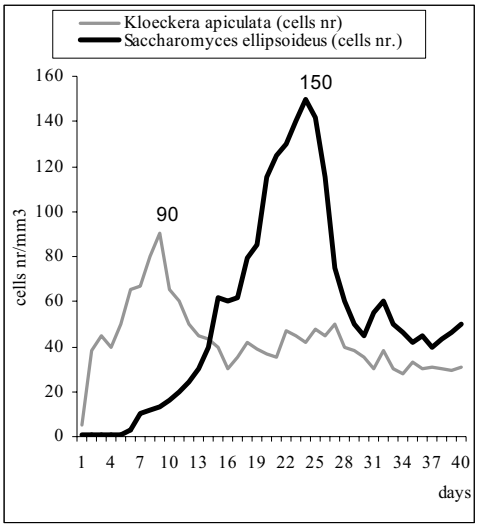

Chart 3 – The cells number evolution at V3 (simultaneous inoculated with Kloeckera apiculata and Saccharomyces ellipsoideus; almost 5 days inhibition for Saccharomyces ellipsoideus)

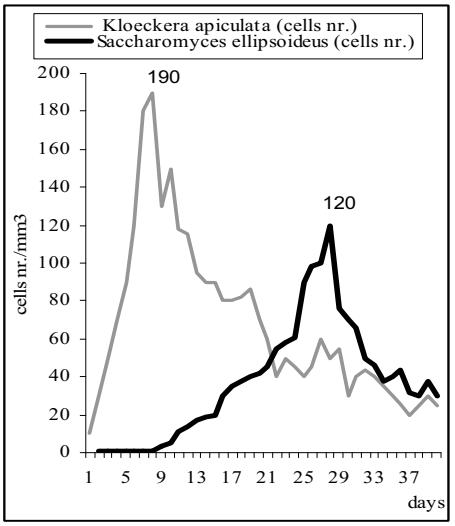

Chart 4 – The cells number evolution at V4 (inoculated with Kloeckera apiculata and after one day with Saccharomyces ellipsoideus; almost 7 days inhibition for Saccharomyces ellipsoideus)

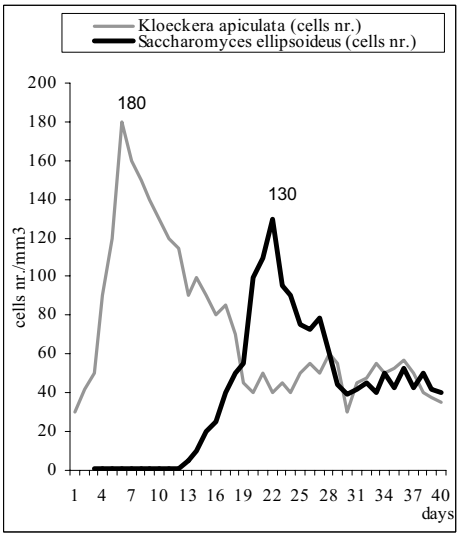

Chart 5 – The cells number evolution at V5 (inoculated with Kloeckera apiculata and after two day with Saccharomyces ellipsoideus; almost 10 days inhibition for Saccharomyces ellipsoideus)

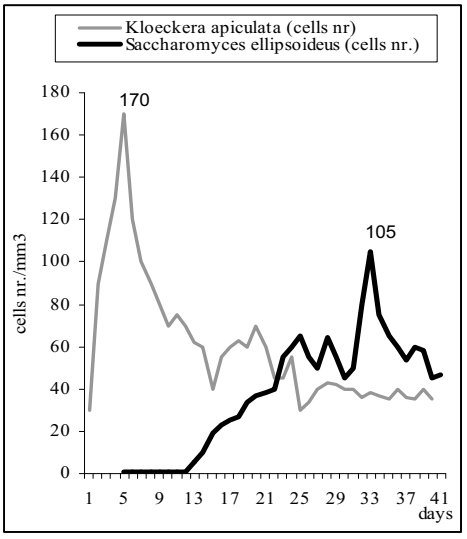

Chart 6 – The cells number evolution at V6 (inoculated with Kloeckera apiculata and after two day with Saccharomyces ellipsoideus; almost 8 days inhibition for Saccharomyces ellipsoideus)

As the strain of Saccharomyces ellipsoideus behavior in mixed inoculated variants (V_3-V_7), there is an inhibition of its multiplication by Kloeckera apiculata, for a number of days, correlated with the_lag time of both species yeast inoculation.

This inhibition is: -almost 5 days for V_3; - almost 7 days to V_4; - almost 10 days for V_5; - almost 8 days for V_6 and almost 5 days for V_7.

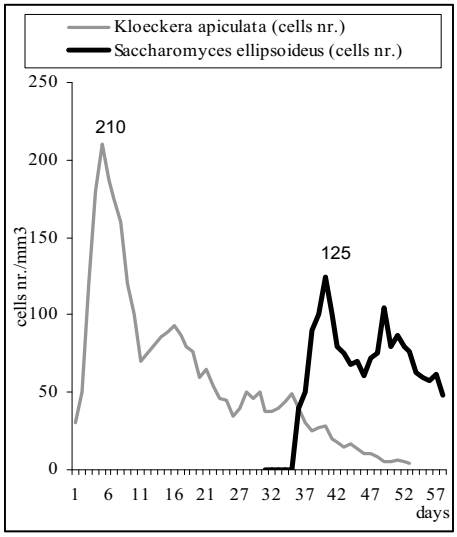

Chart 7 – The cells number evolution at V7 (inoculated with Kloeckera apiculata and after 31 day with Saccharomyces ellipsoideus; almost 5 days inhibition for Saccharomyces ellipsoideus)

The lost in weight curves are different for variants mixed inoculated (V_3-V_7), comparatively with variants inoculated with one species of yeast (V_1 and V_2). If to V_1 and V_2, fermentation curve (CO_2 % release) recorded a single maximum (chart 1 a and chart 2a), the V_3-V_7 variants, it has two maximum (chart 3a, chart 4a, chart 5a, chart 6a, and chart 7a):

- the I-st maximum, came up in the 6-7 day fermentation and is the most species Kloeckera apiculata fermentation product, due to its intense activity in the first days of fermentation;
- the II-nd maximum was in the 26-27 days (V_3), the 31day (V_4), the 27 day (V_5), the 33 day for V_6 and 46 day (V_7) and is generated by Saccharomyces ellipsoideus, after passed the period of inhibition produced by species Kloeckera apiculata.

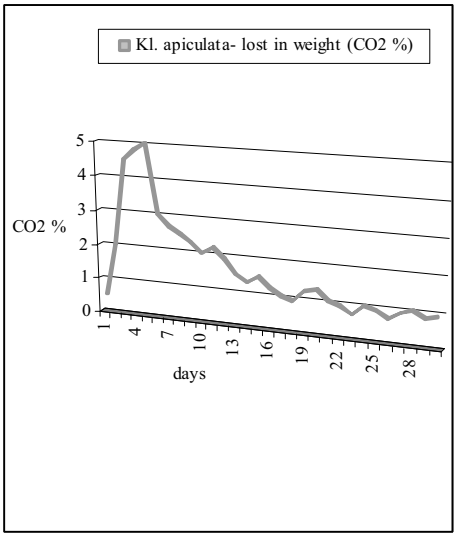

Chart 1a. - The lost in weight at V_1

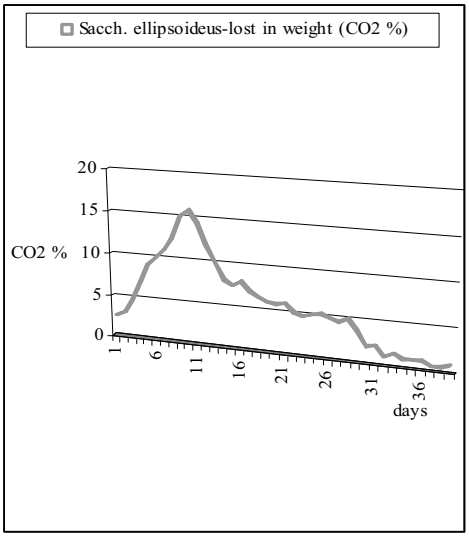

Chart 2a. - The lost in weight at V_2

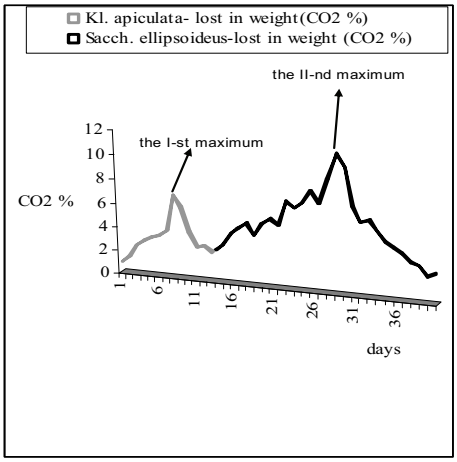

Chart 3a -The lost in weight at V_3

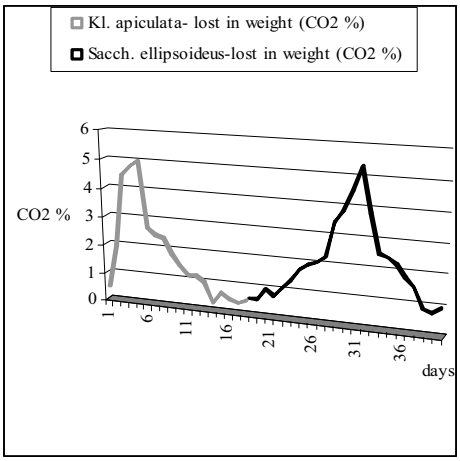

Chart 4a -The lost in weight at V_4

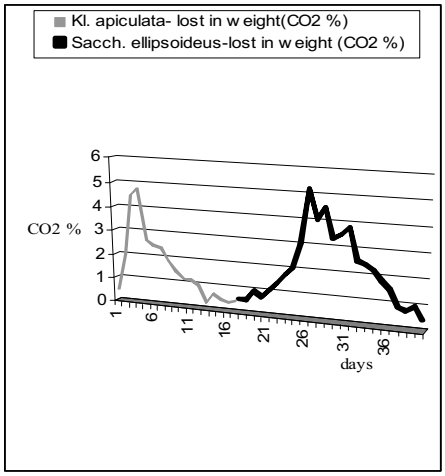

Chart 5a -The lost in weight at V_5

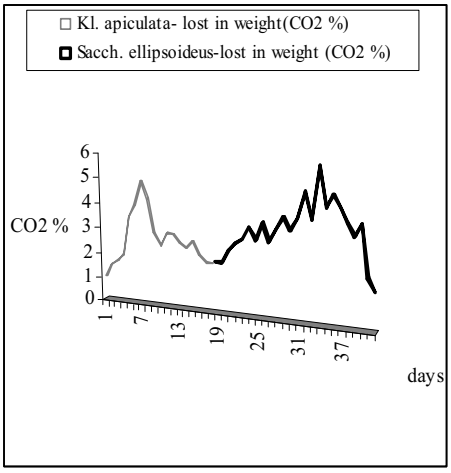

Chart 6a -The lost in weight at V_6

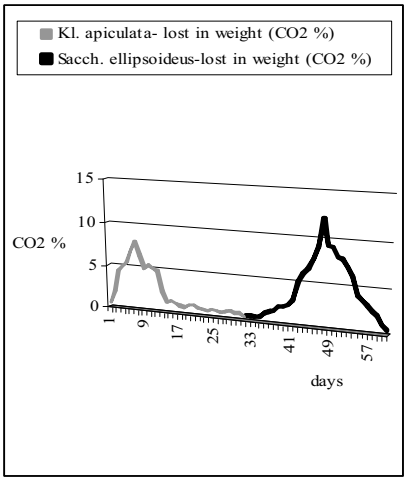

Chart 7a -The lost in weight at V_7

And the chemical analyses of the wines produced, show us the difference of activity between Kloeckera apiculata and Saccharomyces ellipsoideus yeasts species. So, at the variant inoculated with Kloeckera apiculata the alcohol content was 4,85 % vol only, while the variant inoculated with Saccharomyces ellipsoideus have had 11,0 % vol alcohol. The variants mixed inoculated have had an alcohol degree between 8,24-8,82 % vol, closer to V_2, as the time for inoculated of Kloeckera apiculata and Saccharomyces ellipsoideus is shorter (table 1).

The sugars content has a reverse variation comparatively with the alcohol content.

Referring to totale acidity, the low value has V_2, while V_1, has a biggest value.

Volatile acidity has different values for the two yeasts species. Thus, Saccharomyces ellipsoideus in pure culture produce a small quantity of volatile acids (0,43 g/l H_2SO_4-V_2) comparatively with Kloeckera apiculata which exceling by her content in volatile acidity (1,40 g/l H_2SO_4-V_1).

Table 1- The physico-chemical composition of the wines obtained

Sample	Sugar g/l	Alcohol % vol.	Total acidity g/l H_2SO_4	Volat. Acidity g/l H_2SO_4
Must	229,0	-	6,74	-
V_1	128,0	4,85	6,20	1,40
V_2	43,0	11,0	4,80	0,43
V_3	67,3	8,82	6,03	1,04
V_4	67,3	8,75	6,00	1,09
V_5	76,3	8,40	5,94	1,03
V_6	73,6	8,42	6,10	1,06
V_7	74,5	8,49	6,01	1,00
V_8	68,0	8,24	5,97	0,93

CONCLUSIONS

- The apiculate yeasts has the main role to the formation of wine flavours.
- The famous wines, can not be obtained in exclusivity with elliptical yeasts, but by using the spontaneous microflora from the vineyard.
- Kloeckera apiculata yeasts has a big fermentative intensity in the first four-five days, but Saccharomyces ellipsoideus species in the first eight days.
- In the power fermentation period (1-4, 1-8 days) the number of yeasts cells are increase and after this period was not possible to establish a correlation between total yeasts cells number /mm^3 and the fermentation intensity.
- When the samples were mixed inoculated (V_3-V_7), we have seen an mutual inhibition of two yeasts species, correlate with their inoculated moment.
- The lost in weight curves are different to the samples mixed inoculate (V_3-V_7) comparatively, with the samples inoculated with one yeasts species (V_1 and V_2).
- In conclusion, during alcoholic fermentation process, between the two species of yeasts have established negative relationship, of antagonism, which indicated that the Kloeckera apiculata species, by his activity of the metabolites produced in the fermentation medium, unfavorable to the Saccharomyces ellipsoideus species, inhibiting its development and activity for a specified number of days, correlated with their lag time of inoculation.

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