THE RELATIONSHIP ESTABLISED BETWEEN THE GLYCEROL FORMING IN THE WINES AND FERMENTATION CONDITIONS

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Abstract: *The secondary products of alcoholic fermentation, are compounds that appear in variable quantities in addition to alcohol and CO_2 and they are: glycerol, lactic acid, acetic acid, formic acid, substances acetoin, 2,3 butanediol, malic acid, succinic, propionic, citramalic ,dimetilgliceric. Glycerol is formed by glycero-pyruvate fermentation. Glycerol, like secondary product of the alcoholic fermentation, has the greatest importance due to its favourable influence on organoleptic properties of wines. Its synthesis depends upon many factors. This study was conducted under laboratory conditions, using selected yeast strains from the Murfatlar vineyard, viticol Centre Medgidia, belonging to the Saccharomyces ellipsoideus, Saccharomyces italicus and Saccharomyces rosei species. The fermentation medium was Pinot gris must which was inoculated with 1.5 to 1.7 x 10^7 cells/ ml, from the above mentioned yeasts. The objective was to establish the importance of several factors that determine wine glycerol concentration (e.g. must sugar and glycerol, concentration of inoculum, fermentation temperature and yeast species). The glycerol produced was determined by a volumetric method with potassium periodat. The result were: Depending of the grapevine variety and sugar content of the must, glycerol concentration of the grapes varies between 1.70 to 3.02 g/L; The inoculation rate had an influence on the glycerol concentration of wines; The most glycerol was formed in the first 2 to 3 days of fermentation; in this period 80 to 90 g/L of the initial sugar decreased and about 2/3 of the glycerol was formed; The most glycerol was obtained at fermentation temperatures of 20^0C and 25^0C; A fermentation temperature >30^0C leads to a decrease of glycerol formation; Yeast species and the sugar content had the greatest contribution to glycerol formation. Among the strains tested, SE-20, belonging to Saccharomyces ellipsoideus species was notable. This strain produced the highest glycerol concentration in wines (6.50 to 9.80 g/L) and during fermentation did not produce foam.*

Key words: *glycerol, selected yeast, organoleptic proprieties*

INTRODUCTION

The secondary products of alcoholic fermentation, are compounds that appear in variable quantities in addition to alcohol and CO_2 and they are: glycerol, lactic acid, acetic acid, formic acid, substances acetoin, 2,3 butanediol, malic acid, succinic, propionic, citramalic ,dimetilgliceric. Glycerol is an alcohol trihidroxilic, acyclic, saturated in which an -OH group is secondary and the others primary. Small amounts (less than 1 g/litre), glycerol can be found in musts obtained from healthy crops. Larger amounts of glycerol are formed in grapes harvested in supramaturation moment, from sugars, without precise knowledge of the biosynthesis route. Because the glycerol formation occurs simultaneously with the formation of pyruvic acid, fermentation process is called glycero-pyruvate and can be shown by the following chemical equation: $C_6H_{12}O_6 \rightarrow CH_2OH\text{-}CHOH\text{-}CH_2OH + CH_3\text{-}CO\text{-}COOH$

glycerol pyruvic acid

In this way are formed early in alcoholic fermentation 2-3% glycerol.

The researches showed us that indifferent of conditions, by yeasts sugars transformation, results alcohol like main product and glycerol, like main secondary product of alcoholic fermentation process. Approximate 92 % from the sugars quantity from the must is transforming in etanol and 8% in glycerol. The white wines with onctuozity are more appreciated by many consumers.

MATERIAL AND METHODS

The study was made in laboratory and than in production conditions in viti-vinicol Centre Medgidia, situated in Murfatlar vineyard, using selected yeasts belonging to the *Saccharomyces ellipsoideus*, *Saccharomyces italicus* and *Saccharomyces rosei* yeasts, from SC Agerferm laboratory yeasts collection. We mentioned that these yeasts strain were isolated from Murfatlar vineyard.

The fermentation medium was represented by Pinot gris musts which were inoculated with 1.5 - 1.7 x 10^7 cells/ml. It had in view the specification of some factors that determine the glycerol content in the wines (the present of glycerol in the fresch must, the influence of inoculated density, the influence of fermentation temperature, yeast specie and the sugars content of the must).

The glycerol produced was determined by volumetrique method with potassium periodat.

RESULTS AND DISCUSSIONS

First, it was determined the glycerol presence in fresch musts with different sugars contents (table 1).

Table 1

Glycerol content (g/l) of some fresch musts

The variety	Sugars (g/l)	Glycerol (g/l)
Chardonnay	216	1.80
Chardonnay	222	1,95
Muscat ottonel	200	2.30
Muscat ottonel	210	2.70
Muscat ottonel	224	3.02

The results obtainned shown that the fresch must contains differents quantities of glycerol depending of grapes variety and the harvest moment. This analise confirm us the presence of glycerol before the alcoholic fermentation.

For to check the influence of inoculation density on the forming of glycerol there were tested the strains *Saccharomyces ellipsoideus* SE-20, *Saccharomyces italicus* SI-15 and *Saccharomyces rosei* SR-2 in Pinot gris must with 189 g/l sugars and 6,8 g/l H_2SO_4 total acidity.

It had in view the prefermentation period, time of alcoholic fermentation and there were made the main characteristics of wines: alcohol content, residual sugars, total acidity, glycerol (table 2).

Table 2

The influence of the yeasts species and of the inoculated density on alcoholic fermentation

Yeast specie	Inoculated density cells/ml	Preferm. period (hours)	Time of alc. ferment. (days)	Alcohol vol. %	Sugars g/l	Total ac. g/l H2SO4	Glycerol g/l
Saccharomyces ellipsoideus SE -20	7.9 x 10	26	9	11.0	2.0	4.60	8.50
	1.3 x 10	23	8	11.0	2.1	4.80	8.45
	2.5 x 10	19	8	10.8	4.5	3.90	6,50
	5.5 x 10	15	8	10.9	2.7	3.75	9,80
Saccharomyces italicus SI -15	4.4 x 10	53	12	10.5	9.5	4.50	4.72
	3.1 x 10	50	13	10.4	11.2	4.60	5.00
	4.6 x 10	42	11	10.7	6.1	3.90	4.60
	6.5 x 10	39	9	10.6	7.8	4.37	5.90
Saccharomyces rosei SR -2	6.5 x 10	36	11	10.8	4.3	3.90	5.60
	1.2 x 10	35	10	10.8	4.2	4.60	5.03
	5.0 x 10	28	9	10.9	2.6	4.11	6.87
	7.5 x 10	24	8	11.1	0.7	3.85	6.63

The results from the table 2 show that indifferent of inoculate density, the quantity of glycerol of the wines obtained was aproximatively equal within the same specie of yeast.
To establish the period when more glycerol is formed, it was checked the evolution of sugars, alcohol, acetaldehide and glycerol during alcoholic fermentation for SE-20 strain. The results obtained, shown that the forming of glycerol start simultaneous with the multiplication and fermentative activity of the yeasts (fig. 1).

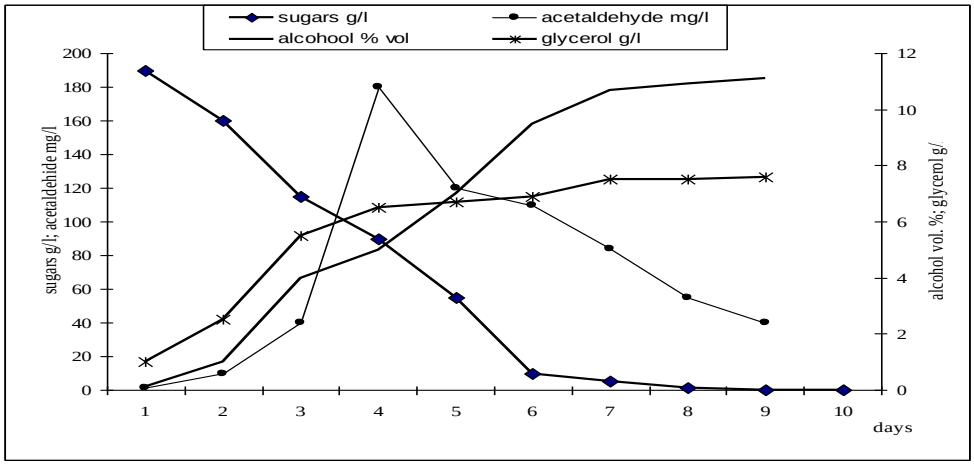

Fig. 1. Evolution of the main must components during alcoholic fermentation

Generally, in the first days of fermentation, the sugars content decreased with 80-90 g/l and the glycerol content that is produced is about 5.6 - 6.0 g/l. Acetic aldehide was not found at the start of fermentation; she was founded after 15 - 30 hours, period in which the

greatest quantity of glycerol was produced in the wines. To the end of fermentation the quantity of acetic aldehyde decreased, till 42 mg/l value. As the acetaldehide content decreased, a small increase of glycerol was registered in the first days of alcoholic fermentation.

It is known that the yeast specie and sugars must content has a big influence in the forming of glycerol process and therefore the strains mentioned before were tasted in the must with different sugars contents 185- 205 - 226 g/l, and fermented at 20^{o} C temperature (table 3).

Table 3

The influence of the yeasts species and must sugars contents on the glycerol content of the wines

Yeast strain	Sugars g/l	The main characteristics of the wines obtained			
		Alcohol vol. %	Sugars g/l	Total acidity g/l	Glycerol g/l
Saccharomyces ellipsoideus SE-20	185	10.8	1.4	3.90	6.50
Saccharomyces italicus SI - 15	185	10.6	4.5	4.05	5.00
Saccharomyces rosei SR-2	185	10.8	1.5	3.67	6.15
Saccharomyces ellipsoideus SE-20	205	11.8	4.0	4.12	7.90
Saccharomyces italicus SI - 15	205	11.6	7.5	3.86	6.30
Saccharomyces rosei SR-2	205	11.9	2.5	3.76	7.11
Saccharomyces ellipsoideus SE-20	226	13.1	3.3	3.87	9.80
Saccharomyces italicus SI - 15	226	13.0	4.9	4.12	7.00
Saccharomyces rosei SR-2	226	13.2	1.6	4.02	7.93

The yeast strains SE-20, SI-15 and SR-2 formed great quantities of glycerol, proportional with sugars musts content.

SI-15 and SR-2 strains have a high foaming degree, unlike the SE-20 strain which does not foam produce.

For knowing the influence of the yeast strain and fermentation temperature on the forming of glycerol, it was tasted the fermentation at different temperature 15^{o} C – 20^{o} C – 25^{o} C (table 4).

Table 4

The influence of the yeast strain and of fermentation temperature on the glycerol content
(Pinot gris must, 215 g/l sugars, 6,30 g/l total acidity, inoculation density $2.1x10^5$ cells/ml)

Yeast specie	Time of alc. ferment (days)	The main characteristics of wines obtained				
		Alcohol vol. %	Sugars g/l	Tot Ac. g/l	Glycerol g/l	Foaming degree
Fermentation temperature = 15° C						
Saccharomyces ellipsoideus SE-20	9	11,95	12	4,20	6,50	-
Saccharomyces italicus SI-15	12	11,8	4,0	3,90	6,85	+++
Saccharomyces rosei SR-2	10	11,8	3,9	4,06	7,00	+++
Fermentation temperature = 20° C						
Saccharomyces ellipsoideus SE-20	8	11,9	2,5	4,50	9,80	-
Saccharomyces italicus SI-15	11	11,7	6,1	4,02	7,08	+++
Saccharomyces rosei SR-2	9	11,8	4,0	3,98	7,10	+++
Fermentation temperature = 25° C						
Saccharomyces ellipsoideus SE-20	8	11,8	4,2	4,50	8,20	-
Saccharomyces italicus SI-15	8	11,7	6,0	4,12	6,86	+++
Saccharomyces rosei SR-2	9	11,8	4,3	4,03	6,80	+++

From the table 4, it results that the period and the way of alcoholic fermentation as well as the glycerol content are influenced directly by the fermentation temperature.

The biggest quantities in glycerol were obtained at 20° C temperature. The increasing of fermentation temperature to 25° C leads to a slight decreasing of glycerol content.

CONCLUSIONS

Depending on of the grapevine variety and sugars content on the must, glycerol concentration of the grapes varies between 1.70 – 3.02 g/.l;

The inoculation rate used in oenologie has not an influence on the glycerol content of wines;

The most glycerol was formed in the first 2 – 3 days of fermentation; in this period 80 – 90
g/l from the total quantity of the sugars decreased and about 2/3 of glycerol was formed from the total content of the wines;

The most glycerol was obtained at fermentation temperatures of 20° C and 25°C;

A fermentation temperature > 30°C leads to a decrease of glycerol formation;

Yeasts specie and the sugar content have the most important contribution in the glycerol formation. Among the strains tested, SE–20, belonging to Saccharomyces ellipsoideus specie was remarked. This strain produced the highest glycerol concentration in wines (6.50 to 9,80 g/l) and does not produce foam during fermentation.

BIBLIOGRAPHY

1. COTEA D. V., SAUCIUC J., COTRĂU A., ANCA MOISE, VICTORIA COTEA, 1982. Influence de quelques facteurs sur la formation du glycerol dans les vins. 4-e Colloque oenologique Franco-Roumain, 21-27 June, Iaşi.
2. COTEA D.V., ZĂNOAGĂ C., COTEA V.V, 2009. Tratat de Oenochimie, vol. II. Editura Academiei Romane, Bucuresti.
3. GANG DU, JICHENG ZHAN, JINGYUAN LI, YILING YOU and WEIDONG HUANG, 2012. Effect of Fermentation Temperature and Culture Medium on Glycerol and Ethanol during Wine Fermentation Am. J. Enol. Vitic. March 2012. Vol. 63:132-138.
4. NIEUWOUDT H., B. PRIOR, S. PRETORIUS, and F. BAUER. 2002. Glycerol and wine quality: fact and fiction. In: Wynboer: a technical Guide for Wine Producers, nov. 2002
5. RIBEREAU-GAYON J., Y. GLORIES, A. MAUJEAN, and D. DUBOURDIEU. 1998. The microbiology of wine and vinification. In: Handbook of Enology", vol. II, 1st edition, John Wiley and Sons Ltd., New York.
6. SEHOVIC D., V. PETRAVIC, and V. MARIC, 2004. Glycerol and the wine industry–Glycerol determination in grape must and wine. Kemija u industriji 53:505-516.
7. ȚÂRDEA C-TIN, 2007. Chimia și analiza vinului. Editura "Ion Ionescu de la Brad", Iași.