QUALITATIVE EVALUATION OF FRESH MARC–RAW MATERIAL WITH VALUABLE COMPOSITION

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Abstract. The present study pursued the possibility of extraction of sugars and other useful components from fresh marc, by diffusion method. The biological material was represented by two types of marc: one from Greaca Vineyard–Wine Research and Production Station and one from Vinalcool Focsani. It was studied the influence of temperature, time, pH and the amount of washing solution on the diffusion of sugars and other useful components. Extraction efficiency was monitored by determining total sugars in the diffusion solutions, and by measuring the volume of solution. Besides the main components which can be found in large quantities: sugars, nitrogen and potassium tartrate, the fresh marc contains, in various concentrations, phosphorus, calcium and magnesium, also. These ingredients are partially passing into the diffusion solution, some remaining in the washed marc. For this reason, the marc continues to remain, even after diffusion, a raw material that can be further used for valorification.

Key words: grape marc, vinification, secondary products

Introduction

The main secondary products of the wine industry are marc and wine yeast.

The marc, obtained as a secondary–product from grape processing, is not exploited on an industrial scale in our country [BUȚU, *et al.,* 2011]. Considered waste, the amount of approx. 60 000 t/year, it is a real ballast for agriculture [BUSTAMANTE, *et al.,* 2008].

Recovery of the fermented marc by distillation and obtaining marc brandy is no longer practical due to marc storage losses, large volumes of marc processed to recover a small amount of alcohol, distillation installation costs *etc*.

Also, classic biotechnological valorification of marc in order to obtain yeast feed on hydrolysed marc is not cost–effective solution due to extremely high production costs.

Concerns of research teams to recover oil, proantocians and polyphenols from marc are only at experimental stage.

Material and methods

As biological material, were used two types of marc: Marc from Greaca Vineyard–Wine Research and Production Station, resulted from processing a mixture of white and red table grapes obtained from continuous press and contained only skin and seeds, the clusters being previously removed and Marc from Vinalcool Focsani, resulted from white wine grapes, containing clusters, skins and seeds. Both marc variants were fresh, unfermented, from healthy grapes and free from mold. Immediately after harvesting these were distributed in plastic bags, in portions of ca. 100g and stored at–12°C in the freezer.

Thus preserved, the samples were the biological material for our experiments.

The proposed experimental variants, intended to study the possibility of extraction of sugars and other useful components of fresh marc by the diffusion method, with the objective of determining optimal processing conditions of this secondary product.

The laboratory experiments aimed to establish the influence of four factors: temperature, time, pH, and the amount of washing solution on the diffusion in the extraction water of the sugars and of other useful components, from the two types of marc [SPIGNO, *et al.,* 2007]. The experiments
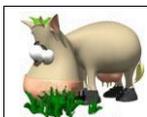
were performed at the following temperatures: 15, 45, 60 and 70°C [MALDONADO, *et al.*, 2010].

Experimental variants at 45, 60 and 70°C were realised by keeping the vessels with marc and solvent on water bath with adjustable temperature.

The extraction duration was 0, 6, 12 and 24 hours. For the experiment at 70°C temperatures, the duration was 15, 30 and 60 min. The pH was studied in three variants: acid (pH 1–2), neutral (pH 7) and alkaline (pH 9–10). Adjustment of pH was done with HCl for acid pH and with NaOH for alkaline pH.

These corrections may be made with H_2SO_4 and Na_2CO_3 respectively.

It should be noted that it was used tap water having pH 7, considering that for industrial processing of marc, the use of softened water as a solvent would impose additional costs.

Of course, when the tap water is hard its treatment is imposed.

Given the possibility of a proper cost–effective valorification of the diffusion solutions by alcoholic fermentation, it was also tried a concentration of sugars by successive washes, in different variants of temperature, pH and the amount of solvent used.

In all experimental variants the samples were taken at the proposed time and the on-going of the extraction was tracked by determination of total sugars.

At each sample used for determination of sugar, in all variants, it was measured the volume of the diffusion solution obtained.

This way it could be calculated the extraction efficiency (g sugar /100 g marc).

The diffusion solutions obtained by successive washing, for the concentration of sugars, as well as fresh marc, before washing and after washing and drying, were characterized in terms of chemical composition, by performing determinations of total nitrogen, protein, phosphorus, potassium, calcium, magnesium, ash, dry matter.

Results and discussion

In the first stage of the research, the laboratory work was performed with marc from Greaca Vineyard–Wine Research and Production Station.

The marc containing only the skin and seeds, being without clusters, did not need a crumbling, because the components were not exceeding 5 mm size. The dry matter content was 53.69%.

The first experiment was followed by extraction of sugar from the fresh marc by tap–water diffusion at a temperature of 15°C, in three variants of pH: 1–2, 7, 9–10.

Times of action of the marc solvent were: 0, 6, 12, 24 h.

At 0h was marked the result of the extraction at simple wash of marc with water extraction and simultaneous flow of the solution obtained (without stationing).

The report marc/water was 1:2, and the results obtained are listed in *Table 1*.

Table 1.

Quantity of sugars extracted from marc at 15°C

Time	Total Sugar (g%ml)					
(h)	Sample	pH=7	Sample	pH=1–2	Sample	pH=9–10
0	M1	0.46	M2	0.49	M3	0.98
6	1	1.33	4	1.42	7	1.79
12	2	1.53	5	1.61	8	1.87
24	3	1.71	6	1.84	9	1.49

By comparing the data, it can be seen that at the simple wash of the marc with

water (0 h), the sugars obtained are in very small quantity, the values recorded at pH 7 and pH 1–2 are about 0.5 g% ml, while at pH 9–10 the quantity is double (0.98 g% ml).

At the diffusion times 6, 12 and 24 hours, the quantities of sugar extracted were close, but not exceeding 2g%ml of solution.

Regarding the extraction efficiency (*Table 2*) and the sugar obtained for 100 g of marc, the best results were obtained at pH 7 and 1–2, namely, 2.74 g and 2.94 g sugar per 100 g marc.

Table 2.

Sugar extraction efficiency at 15°C

Time	Efficiency (g sugar at 100g marc)					
(h)	Sample	pH=7	Sample	pH=1–2	Sample	pH=9–10
0	M1	0.57	M2	0.86	M3	1.97
6	1	2.19	4	2.30	7	2.36
12	2	2.45	5	2.58	8	2.43
24	3	2.74	6	2.94	9	2.23

For the next experiment, the temperature was 45°C, keeping the same work variants.

The results are listed in *Table 3*.

Table 3.

Quantity of sugars extracted from marc at 45°C

Time	Total Sugar (g%ml)					
(h)	Sample	pH=7	Sample	pH=1–2	Sample	pH=9–10
0	M1	0.79	M2	0.68	M3	0.68
6	1	1.68	4	2.02	7	1.96
12	2	2.08	5	2.08	8	2.09
24	3	2.17	6	2.13	9	2.11

At 0 h, the sugars extracted by washing the marc were slightly higher than those obtained under the same conditions at 15°C.

Regarding the extraction at 6, 12 and 24 h, the values obtained at all pH variants, were significantly higher than those recorded under the same conditions at 15°C, beibg over 2g sugar at 100ml.

Extraction efficiency (*Table 4*) was 30 to 40% better, achieving values between 3.7 to 3.8 g sugars per 100g marc.

Table 4.

Sugar extraction efficiency at 45°C

Time	Efficiency (g sugar at 100g marc)					
(h)	Sample	pH=7	Sample	pH=1–2	Sample	pH=9–10
0	M1	1.03	M2	1.12	M3	0.82
6	1	2.64	4	3.17	7	3.08
12	2	3.54	5	3.54	8	3.55
24	3	3.80	6	3.68	9	3.69

In *Table 5* are recorded the data obtained at a different extraction

temperature, i.e. 60°C, extraction performed under the same experimental variants of diffusion times and pH.

Quantity of sugars extracted from marc at 60°C

Time	Total Sugar (g%ml)					
(h)	Sample	pH=7	Sample	pH=1–2	Sample	pH=9–10
0	M1	1.01	M2	0.93	M3	0.87
6	1	2.89	4	2.88	7	2.32
12	2	3.24	5	3.52	8	2.13
24	3	1.77	6	1.99	9	1.68

At this temperature, the extraction panel is different from that obtained at 15 and 45°C.

Right from the time 0 h, the sugars in the washing water were in larger quantities (about 1g in 100ml).

The maximum values of sugars were registered at 12 hours, at pH 7, and 1–2, i.e. 3.24 g in 100ml, respectively 3.52 g in 100ml of diffusion solution, then, at 24 h the sugars extracted decreased under 2g to 100 ml.

At the temperature of 60°C was recorded the best extraction efficiency, 4.22 g sugar at 100g marc at12 h for pH 1–2 (Table 6).

In another stage of research we tried a concentration in sugars of the diffusion solution, by washing successively several samples of 50 g of marc, in two variants, at pH 7 and 1–2.

Sugar extraction efficiency at 60°C

Time	Efficiency (g sugar at 100g marc)					
(h)	Sample	pH=7	Sample	pH=1–2	Sample	pH=9–10
0	M1	1.55	M2	1.69	M3	1.14
6	1	3.93	4	3.97	7	3.06
12	2	3.24	5	4.22	8	2.47
24	3	1.98	6	2.19	9	1.68

Over the first quantity of fresh marc were added 100 ml solution at 60°C and were left for 12 h for diffusion.

After this time, the solution was drained, the volume was measured and then completed with 100 ml water, and leaving the diffusion over a new stack of marc for another 12 h (this can be considered the first concentration).

Three such concentrations were performed, and the results are shown in *Table 7*.

Sugar concentration at 60°C

Sample	pH	Total Sugar (g%ml)			
		12 h	24 h	36 h	48 h
1	7	2.53	2.41	3.10	3.22
2	1–2	2.67	2.68	2.98	4.23

From the analysis of the data presented, it can be seen that after three such concentrations, there was a maximum amount of sugars extracted from sample 2 (pH 1–2) respectively 4.23 g% 100ml.

In *Table 8* are listed the values for extraction efficiency, and the maximum

value, 3.80g sugar at 100g marc, is recorded for version 2.

Table 8.

Extraction efficiency at sugar concentration at 60°C

Sample	pH	Efficiency (g sugar at 100g marc)			
		12 h	24 h	36 h	48 h
1	7	2.52	3.10	3.38	2.90
2	1–2	2.40	2.68	2.94	3.80

The conclusion that emerged from this experiment was that, although the value of 4.23 g sugar % ml is higher than that obtained by only diffusion, it was not a sum of the sugar of the three stacks of marc.

The explanation is the essence of the phenomenon of diffusion: the direction of movement of the substance in a solvent is realised from higher concentration to lower concentration.

As diffusion solution enriched in sugar, the movement took place in the opposite direction, from solution to marc, tending to equalize the concentrations.

To check whether this was the real explanation, the experiment was repeated, this time with a single value of pH, at the extraction temperature of 45°C.

Each stack of marc was subjected to diffusion with the volume of the solution obtained from previous marc washing. The scheme is shown in *Figure 1*.

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0 hours      50 g marc + 200 ml. water
             ↓12 hours difusion
12 hours     50 g marc + 180 ml filtrate
             ↓12 hours difusion
24 hours     50 g marc + 150 ml filtrate (first concentration)
             ↓12 hours difusion
36 hours     50 g marc + 110 ml filtrate (second concentration)
             ↓12 hours difusion
48 hours     80 ml filtrate (third concentration)
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Figure 1. Working scheme for sugar concentration at 45°C

Sugars were determined only once, at the end of the experiment, and the recorded value was 5.05 g% ml.

Although practically it was performed a concentration of the sugars in the diffusion solution (5.05 g compared to 4.23 g), the extraction efficiency was low– 2.02 g sugar per 100 g marc, lower than in the previous experiment (3.80 g sugar per 100 g marc).

In this experiment either, the concentration was not realized by sum of the amount of sugars in the stacks of marc subjected to extraction, but the concentration complies to the diffusion law and was influenced by the ratio marc/water.

In this case, the ratio was 1:1, while in the previous experiment was 1:1.5.

By reducing the amount of water it was obtained a concentration of sugars in the washing solution, but decreased the extraction efficiency, while increasing the amount of water increased the efficiency of extraction, but the diffusion solution was more diluted.

The experiments continued using the biological material from Vinalcool Focsani, the marc containing clusters, skins and seeds, as well as a larger amount of must (dry matter was 31.24%).

Because the marc contained clusters; this was cut for the equalization of components, resulting a better diffusion, as well.

In *Table 9* are presented the quantities

of sugar obtained by extraction at 60°C, at diffusion times 0, 6, 12 and 24 h. It was used three variants of pH: neutral, acid and alkaline.

It was chosed to perform the experiments at the temperature of 60°C, because at this temperature were obtained the best values, both for sugars and extraction efficiency for the marc from Greaca.

Table 9.

Quantity of sugars extracted from marc at 60°C

Time	Total sugar (g%ml)					
(h)	Sample	pH=7	Sample	pH=1–2	Sample	pH=9–10
0	M1	2.30	M2	2.52	M3	1.88
6	1	4.35	4	4.81	7	3.20
12	2	6.77	5	6.89	8	3.45
24	3	3.09	6	3.11	9	2.89

At 12 h, at pH 7 and 1–2 were extracted the largest quantities of sugar, namely 6.77 g% ml, respectively 6.89 g % ml. For these variants was registered a better extraction efficiency–6.77, respectively 7.43 g sugar per 100g marc (*Table 10*).

Table 10.

Efficiency of extraction at 60°C

Time	**Efficiency** (g sugar at 100g marc)					
(h)	Sample	pH=7	Sample	pH=1–2	Sample	pH=9–10
0	M1	2.40	M2	2.93	M3	2.10
6	1	6.22	4	6.45	7	5.56
12	2	6.77	5	7.43	8	4.47
24	3	3.53	6	4.01	9	2.89

From the ccomparative analysis of the data obtained it can be seen that, due to the high degree of soaking with most of the marc from Focsani (humidity 68.76%), the sugars obtained by diffusion, for all variants, are significantly higher than those achieved under the same working conditions, for the marc from Greaca (46.31% humidity).

There is yet another explanation of this situation, namely that the sugars values from the marc depend on their concentration in the grapes from which the marc was obtained.

It is well known that the grapes from Vrancea area, especially the wine grape varieties reach appreciable quantities of sugar towards the end of ripening, while Greaca area has nobler table grapes that do not reach high concentrations of sugar.

Next, the experiment performed a parallel concentration of sugars, using the two types of marc, according to the protocol presented in *Tables 11, 12*.

Table 11.

Working protocol for sugar concentration–Greaca

Time (h)	pH=1–2		pH=7	
	Inițial volume (ml)	Final volume (ml)	Inițial volume (ml)	Final volume (ml)
0	400	350	400	350
12	350	320	350	300
24	320	280	300	250
36	280	240	250	220
48	–	240	–	220

Table 12.

Working protocol for sugar concentration–Focşani

Time (h)	pH=1–2		pH=7	
	Inițial volume (ml)	Final volume (ml)	Inițial volume (ml)	Final volume (ml)
0	400	390	400	390
12	390	390	390	390
24	390	390	390	390
36	390	420	390	420
48	–	420	–	420

The measurements of sugar extracted were made once, at the end of the experiment, in the diffusion solution finally obtained.

In the same working conditions, for the marc from Greaca, because of lower humidity (46.31%), the quantity of diffusion solution, obtained on the ongoing of the experiment and at the end, was lower than the one for the marc from Focsani.

The extracted sugars were 4.16 g % ml for pH 1–2 and 4.62 g % ml for pH 7, with extraction efficiency of 2.50, respectively 2.54 g sugar per 100g marc.

By comparison, due to high humidity (68.76 %), for the marc from Focsani the diffusion occurred, in all phases, in a large amount of liquid, the final volume being even slightly larger than the original.

Quantity of sugars from the solution for diffusion in Focsani marc was, at pH 1–2 of 9.86 g % ml, and at pH 7 of 8.93 g % ml, with extraction efficiencies of 10.35, respectively 9.38 g sugar per 100 g marc.

In the last phase of the research was studied the possibility of extracting sugar from the fresh marc at a temperature of 70°C.

The two types of marc were subjected to diffusion for 15, 30 and 60 minutes, with water at pH 1–2 and temperature of 70°C.

The ratio marc / water were 1:2.

Diffusion results, expressed in quantities of sugar obtained and extraction efficiency, and are listed in *Table 13*.

Table 13.

Sugar extraction at 70°C

Time (min.)	Marc from Focşani		Marc from Greaca	
	Total sugar (g%ml)	Efficency (g sugar at 100g marc.)	Total sugar (g%ml)	Efficency (g sugar at 100g marc)
15	4.65	6.50	2.29	3.38
30	3.81	5.34	2.09	2.92
60	3.12	4.36	1.88	2.78

It can be seen that, at a temperature of 70°C, the largest quantities of sugars are extracted in the first 15 min., gradually decreasing to 30 min. and 60 min. for both types of marc.

The best extraction efficiency was recorded at 15 min. for Focsani, 6.50 g sugar per 100 g marc and respectively 3.38 g sugar per100g marc, for Greaca.

It is noted that the values obtained from measurements of total sugars in the first 15 min. of extraction at 70°C, for both types of marc, are comparable to the results achieved in the experiments at 60°C, at 24 h of diffusion, but were lower than those recorded at 12 h of diffusion.

In the same conditions of temperature (70°C), at pH 1–2, it was experimented the influence of quantity of solvent on sugar extracted and extraction efficiency.

The ratio marc/water was this time 1:4, contact time 1 h.

Table 14.

Influence the brand/water on the extraction 70°C

Sample	Total sugar (g % ml)	Efficiency (g sugar per 100g marc)
Greaca	1.62	5.51
Focşani	2.86	9.72

Comparing the results (*Table 14*)., it can be seen that, by increasing the volume of water, diffusion was favored, expressed in a better efficiency both for the marc from Greaca (5.51 g sugar per 100g marc) and the one from Focsani (9.72 g sugar per 100 g marc).

The results are much better than those obtained at 1h, under the ratio marc / water 1:2, namely for Greaca 2.78g sugar per 100 g marc and Focsani 4.36 g sugar per 100g marc.

The sugars contained in the diffusion solutions are comparable to those extracted at 1 h in the previous experiment, but lower than values at 15 min. of extraction

For a complete characterization, the measurements were performed for N total, P, K, Ca, Mg and dry matter.

The samples used were for fresh

marc, washed and dryed marc, and for the diffusion solution, and the results are listed in *Tables 15, 16, 17*.

Table 15.

Chemical composition of the marc from Greaca

Sample	A*	B*
N total (g%g. d.m)	3.03	1.85
Protein (g%g. d.m)	18.99	11.60
P (g%g. d.m)	0.08	0.005
K (g%g. d.m)	0.84	0.54
Ca (g%g. d.m)	0.63	0.51
Mg (g%g. d.m.)	–	–
Ash (g%g. d.m.)	3.54	3.23
D. mater (g%g.sample)	53.69	92.87
A* = fresh marc; B* = washed and dryed marc;		

Table 16.

Chemical composition of the marc from Focşani

Sample	A*	B*
N total (g%g. d.m)	5.95	2.12
Protein (g%g. d.m)	37.29	13.28
P (g%g. d.m)	0.26	0.04
K (g%g. d.m)	2.08	0.83
Ca (g%g. d.m)	1.15	0.53
Mg (g%g. d.m.)	0.05	0.01
Ash (g%g. d.m.)	6.66	3.38
D. mater (g%g.sample)	31.24	93.15
A* = fresh marc; B* = washed and dryed marc;		

Table 17.

Chemical composition of the diffusion solution from marc

Sample	N total (g%ml.)	Protein (g%ml.)	P (g%ml.)	K (g%ml.)	Ca (g%ml.)	Mg (g%ml.)
Greaca	0.09	0.58	0.007	7.0	0.43	–
Focşani	0.11	0.72	0.24	9.8	0.9	0.49

Comparing the results shown in *Tables 15* and *16* it can be seen the differences between the constituents of the two types of analyzed marc.

The marc from Focsani has higher values than the one from Greaca, for all components: total nitrogen and calcium is 2 times higher, phosphorus is 3 times higher and potassium is 2.5 times higher.

Regarding the chemical composition of the two diffusion solutions obtained from marc from Greaca and Focsani (*Table 17*), it can be seen that the total

nitrogen values are close (0.09 and 0.11), while concentrations in minerals are higher in the solution obtained from the marc from Focsani.

It can be concluded that, besides the main components which can be found in large quantities: sugars, nitrogen and potassium tartrate, fresh marc contains, in various concentrations, phosphorus, calcium and magnesium, also [PATTI, *et al.,* 2009].

All these ingredients are passing into the diffusion solution, but not entirely,

some remaining in the washed marc. For this reason, the marc continues to remain, even after diffusion, a raw material that can be further used for valorification.

Both sugars and other components are found both in fresh marc and the solution obtained by diffusion, in different concentrations, depending on many factors related both to the quality and origin of marc and diffusion conditions.

If we cannot influence the composition of marc, we can control the diffusion conditions.

Knowing this phenomenon, we can lead the process of extraction of sugars and other useful components according to the destination that we shall give to these diffusion waters.

From the above data it can be said that the solutions obtained by diffusion cannot be standardized, but they have a chemically valuable composition and their valorification possibilities is scientifically interesting to be further studied.

Conclusions

The present study pursued the possibility of extraction of sugars and other useful components from fresh marc by diffusion method.

The experiments used as biological material two types of marc: one from Greaca Vineyard–Wine Research and Production Station and one from Vinalcool Focsani. Both marc variants were fresh, unfermented, and were portioned and stored in plastic bags in a freezer at–12°C. It have been proposed several experimental variants during which it was studied the influence of temperature, time, pH and the amount of washing solution on the diffusion of sugars and other useful components.

Extraction efficiency was monitored by determining total sugars in the diffusion solutions, and by measuring the volume of solution, thus it could be calculated the extraction efficiency.

It was established that, at the same time of contact of the marc with the solvent, at lower temperatures (15 and even 45°C), the sugars in solution (g% ml) as well as the extraction efficiency (g. sugar/100g.marc) are lower than those achieved at a temperature of 60°C.

The acid pH (1–2), and the neutral one (7) have a positive influence on the extraction, being preferable to the alkaline one. The maximum amounts of total sugars was obtained at a temperature of 60°C at 12 hours of diffusion, at pH 1–2, both for the marc from Greaca (3.52 g. sugar / 100 ml. sol.) and for the one from Focsani (6.89 g. sugar/100ml. sol.).

In the same conditions was recorded the best extraction efficiency, namely 4.22 g sugars/100 g marc for Greaca and 7.43 g sugars/100 g marc for Focsani.

By successively washing several doses of marc with the diffusion solution obtained from previous marc, in order to concentration of sugars, in final solution was obtained 4.62 g. sugar % ml for the marc from Greaca and 9.86 g sugar % ml of solution for one from Focsani.

Under given experimental conditions, the results obtained are not a summation of the amount of sugar from the doses of washed marc, but is explained by the essence of phenomenon of diffusion, the movement of molecules of substance being realised from the concentrated solution towards the diluted one, until equalization.

The amount of solvent (ratio marc / water) influences the amount of sugar in that, in a larger quantity of water, the extraction is favored and is obtained a better efficiency, but the concentration of sugars in the solution of diffusion is low, and vice versa. Marc humidity, respectively its soaking degree with must, influences the amount of sugar extracted by diffusion. The marc from Focsani, with a humidity of 68.76 %, at all experimental variants, the quantities of sugar in solution were higher than

those obtained from the marc from Greaca, with a moisture content of 46.31 %. The values of sugars from marc depend on sugar concentration in grape from which the marc originates.

The sample from Focsani was obtained from wine grapes with higher sugar concentration, while the Greaca sample came from table grapes, with a lower concentration of sugar.

Diffusion solutions obtained by successive washes, as well as the marc before washing and the one after washing and drying, were characterized in terms of chemical composition, performing the determinations of total nitrogen, phosphorus, potassium, calcium, magnesium, ash, dry matter.

For all components the marc from Focsani presented higher values than that from Greaca: total nitrogen and calcium was 2 times higher, phosphorus was 3 times higher, potassium 2.5 times higher. In the diffusion solutions the total nitrogen values were close (0.09 and 0.11 g% ml) while mineral elements concentrations are higher in the solution obtained from the marc from Focsani.

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