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STUDIES ON THE POSSIBILITY OF RECOVERY OF SECONDARY PRODUCTS RESULTING FROM THE PROCESS OF VINIFICATION

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Abstract. The problem of valorification of wine products was treated differently over time. In the past it was regarded as a minor issue, but today occupies an important place both in researches issues, as well as in economics, because of the global crisis of raw materials and energy, and because of environmental issues involved, too. Under these conditions, research in biotechnology that brings new solutions and can contribute in solving any of these problems is of particular interest. This task is required therefore as a necessity of the economy, with the intention to bring into attention subproducts of a definite value that have a special attention of the specialists abroad, but who are considered waste in our country and treated as such. In this work are presented and analyzed the recovery directions in this field worldwide, as well as research progress in our country.

***Keywords*:** vinification, secondary products, wine yeast, marc

Valorification of the secundary products of wine worldwide

The problem of obtaining and using the secundary products of the wine shows the various practical and economic aspects and directions, depending on the level of technology of this industry and economic needs of the country. Also, there are large differences between the technical levels from small to industrial enterprises.

In Italy, while in previous years only part of the products were recovered, as the fields of wine were far away from wine processing facilities, today the situation has changed due the location of such facilities in different areas.

The secundary products of wine are mostly valorified on an industrial scale.

Exceptions to this rule are appreciable quantities of marc and, to a lesser extent, yeast, processed and artisan scale or semiindustrial. Following an ancient tradition, in some regions of Northern Italy (Italy septenrionala) marc are used to produce the distillate which was once typical for these regions, but today is spreading fast in the rest of Italy. We are talking about "Grappa"', whose consumption has gone from 4 million bottles in 1959, tens of millions today, as a distillate with an alcohol concentration of 43^0–45^0 and is sold either fresh, after distilling because of the pleasant bouquet or in a lesser extent, after 2–3 years aging. Marc distillation to obtain distillate Grappa "type" takes place in plants with small-scale nature, traditional installation, with discontinuous operating and in industrial distilleries with continuous process capable to 40–100 tons of marc in 24 hours [BIRD, 2005; AMERINE, 1972].

Marc exhausted of alcohol is further treated with acidified water and with sulfuric acid in order to obtain tartrate and then with calcium carbonate, to obtain calcium tartrate that is separated by centrifugation or decanting. They are sold to industries specialized in obtaining pure tartaric acid or potassium hydrogen.

Marc is exhausted of tartrate and alcohol and is dried to 82–92%, ground and granulated to be guided to the feed industry. From 100 kg of wet marc are achieved on average of 16–20kg of granulated marc. Analysis of a sample of the dry marc gave the following results (% dry matter):

- moisture 11.28;
- crude protein..... 13.98;
- crude lipids 7.52;
- crude fiber 28.97;
- ash..... 2.47;
- non–nitrogenous extracts ..... 47.06.

To a lesser extent, red grape marc is used to extract anthocyans (oenocyanin), a complex of colored pigments contained in the husks of black grapes of *Vitis vinifera*.

Extraction procedures are based on

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the use of sulfur dioxide, the most pervasive being the Carpentier process. Marc is first subjected to maceration in sulfur dioxide solution where diffusion is achieved.

This way is obtained picket sulfur that is first cleared by defecation, and then concentrated at low temperature (around 40^0C). A good part of the oenocolorantul produced in Italy is exported to the U.S.A.

Another product that sparks a particular interest in Italy is the grape seeds, whose oil, once having a minimal commercial value, are now unexpectedly re–evaluated, given the destination of his special dietary purposes. In Italy 2–3 hundreds of thousands tons of grape seeds are processed that give more than 25 thousands tons of oil.

In recent years, grape seed oil has seen a growing demand from consumers, being particularly recommended due to high content of unsaturated fatty acids (about 1.85%). The characteristic of this oil is its exceptional content of linoleic acid (55–65%). Gas–chromatographic analysis of grape seed oil has provided the following results:

- myristic acid ..... traces;
- palmitic acid 8.40%;
- palmitoleic acid 0.30%;
- stearic acid 3.90%;
- oleic acid..... 20.50%;
- linoleic acid 65.40%;
- arachitic acid..... 1.50%;
- eicosenoic acid ..... traces;

Other samples gave the following values:

- linoleic acid .....until 70%;
- density at 20^0C..... 0.913–0.937;
- refractive index nD 20^0C..... 1.473–1.477;
- putere calorica 9.540cal/g;
- iodine index 94–131;
- saponification index .....92–97.

Another reason that increases the dietary value of grape seed oil consists of notable content of tocopherol (vitamin E): 90–135 mg/100g oil.

Grape seed oil extraction is done by pressure or solvent extraction, the second method is used almost everywhere nowadays [SCHNEIDER, 1987; ARAGON, 1998; VOLSCHENK, 1997; SANTOS, 2008].

The crude oil extracted is successively subjected to distillation, which includes the following operations:

a) neutralization with alkali;
b) discoloration with charcoal or bleaching earth;
c) eliminate of odors under vacuum at 250^0C;
d) extract of margarine, which consists of cooling at 5^0C for precipitating solid glycerides, followed by a filtration for separating liquid glycerides.

The flour obtained from oil extraction from seeds and which was once almost exclusively pressed into briquettes for use as fuel, is now directed to fodder industry, because of the composition of the types of flour extracted from the seeds:

Composition	Protein flour (grind) (%)	Whole flour (defibrata) (%)
moisture	10	10
crude protein	26	12
digestible protein	18	–
fats	6	1,5
crude fiber	24	48
ash	5	3
non–nitrogenous extracts	39	35,5

In terms of wine yeast, almost all, except those that come from the fining (clearing treatments) are used to extract tartrate. In equipped and authorized companies for distillation, from wine yeast is obtained alcohol of $96–97^0$ with a good flavor, almost no methanol, but containing large quantities of amyl oil [HORNSEY, 2007].

Wine stone deposited on the walls of large cement tanks is recovered by using flame heating. In France things are different in terms of marc and wine yeast valorification.

French and EU regulations require wine makers to deliver a fixed quantity of pure alcohol to state monopoly and this is why the secundary products resulted from vinification are designed primarily for the manufacture of alcohol or spirits distillation.

Special attention is given to the seeds separated from marc after the exhaustion of alcohol. Large quantities of grape seeds (65,000–110,000 tons) are treated in private facilities attached to the distilleries.

Average oil content of these seeds is around 10%, obtaining a diet oil little desiccant or a very desiccant oil for soap industry.

Besides oil, the production of cakes is important due to their composition. After P.

Flanzy, their composition is as follows:

- water 12%;
- cellulose 45%;
- fat 4%;
- starch and sugar.....24%;
- protein 9%;
- tannin .....7%.
- nitrogen 1%;
- phosphoric acid 1.5%;
- potassium.....2%.

Using these cakes as feed for animals and for the soil fertilization is thus perfectly justified. Recovery of wine yeasts is done by filtration or centrifugation.

Fluid obtained (60–70% by weight of fresh yeast) is supplied to the distilleries for the manufacture of rectified alcohol, and solid waste is delivered to the tartar manufactures. In France, outside the normal use of wine the secundary products listed so far can be added the recovery of amyl alcohol and glycerine.

Oenocianins do not present any interest becouse the use of these pigments in wine production is prohibited by law.

In Spain, another country with a developed wine industry, the secundary products have been reviewed by creating associations, equipped with modern processing plants.

The superior yield obtained, the improved quality of products manufactured and sold in large quantities have strongly influenced their valorification.

After analysis in Viticulture and Enology Station of Requena, the compositionof marc (marc separate from clusters of grains % dry matter) is the following:

Composition	Sample 1 (% s.u.)	Sample 2 (% s.u.)
moisture	53,35	51,66
nitrogen	1,90	1,96
protein matters	11,88	12,25
tartrate	2,81	2,21
tannin	1,36	2,06
alcohol	4,50	3,05
ash	5,24	4,30

Marc is used fresh, without prior digestion or completely fermented, in bins called "briseras" or "pozos".

The cake is cooked fresh or fermented picket, from which is extracted tartrate, too.

After processing these are sold tartaric acid factories.

Another application is their use as animal feed, with the following chemical composition:

Composition	Distilled	Undistilled
nitrogenous matters	11,06	0,56
non–nitrogenous matters	56,85	54,09
cellulose	12,80	8,15
equivalent barley/kg marc	749g	719g

The marc exhausted of alcohol is often varlorificated as organic fertilizer or as fuel in the distillation. The seeds of grapes are also valued, in Spain there are companies specializing in oil–solvent extraction (yields ranging from 10–16%). The average composition of grape seeds:

- water.....38.7%;
- fats.....14.2%;
- crude fiber.....21.2%;
- insoluble hydrates.....12.0%;
- tannin 7%;
- ash .....0.7%.

The obtained oils have the following caractheristics:

- density.....0.925;
- freezing point.....12.60;
- iodine index 132;
- saponification index.....195;
- unsaturated fatty acids 84%;
- color–fractometrical deviation 3.23;
- Heidenneichs reaction .....orange–red.

Seed oil is intended for consumption (when mixed with other oils), or to be used for industrial purposes (lubricants, soaps, paints). Cakes left after extraction is used mainly as animal feed. Wine yeasts are generally used to extract alcohol and tartrate.

Another European country with a strong wine industry is Yugoslavia, which specifically exploit marc and wine yeast.

Fresh or fermented marc is treated with water for alcohol and sugar diffusion. This solution is used to produce spirits by distillation, and exhausted marc is used in the production of organic fertilizer or fodder.

In Yugoslavia yeast is distilled in a proportion of 90%, either alone or in mixture with marc and only 10% is used for the production of tartaric acid.

Vinification units recover the stone wine and sell it to the wine factories that are

producing tartaric acid, used in the wine industry, chemical industry and pastryindustry.

In Argentina, marc, seeds, and yeasts are evaluated on an industrial scale, while on rural–scale or artisanal the fermented marc is 100% embedded directly into the ground improving its structure.

In all cases marc is fully recovered, given that the operations of distillation, separation of tartrate and seeds are succesive and common for all distillation centers. Average yield of calcium tartrate for 100 kg marc is reaching 3%. From this raw material–calcium tartrate–is obtained 90% tartaric acid.

This is mainly used in the oenological industry or used in non–alcoholic beverage, pharmaceuticals and in pyrotechnics.

Given that Argentina is a country rich in animal feed, cake is not valued in this regard. After exhausting alcohol, the seeds extracted from moist marc are fully used in oil extraction using solvents: light petroleum, gas *etc*., residues from the extraction will not be recovered. Pressed and evaporation dried wine yeasts, with a dry matter content of 49.7%, have the following average composition (% DM):

- nitrogenous substances 60.97g;
- cellulose 29.25g;
- fatty substances.....2.75g;
- minerals 7.03g;

Hydrogen concentration ranges from 25–325. They are used 100% for alcohol and hydrogen extraction, tartrates yield ranging from 20–30kg to 100kg dry yeast.

In Germany is not practiced a valorification on industrial scale of marc, grape bunches, husks or seeds.

Only a small fraction of yeast obtained are distilled in agricultural enterprises, thereby achieving "Hefeschnaps" (spirit of yeast), which is sold as vodka consumption. The marc serves exclusively, and without any treatment, composting for use as fertilizer, its exploitation is not profitable otherwise.

In industrial enterprises only a small portion of wine yeast is transformed into alcohol, the remainder being used to obtain tartaric acid and salt grape Seignette.

Grape seeds are not separated from the marc, serving with the skin, exclusively to the manufacture of compost.

From the manufacture of alcohol from the liquid yeasts result as by–product enanthic ether (oil yeast), a mixture of alcohols with relatively high boiling point and other volatile aromatic substances used in the perfume industry and chemical industry. Hungarian specialists have successfully performed experimental work to obtain amino acid preparations from the yeast.

Amino acids, the secundary products of the hydrolysis of proteins, are forming almost 75% of the total nitrogenous substances of yeast precipitation. From 100kg of yeast can be obtained 1.5kg amino acids by using ions. In pure form were obtained: tyrosine, asparagine, glutamic acid and others.

In many research institutes in various republics of the former USSR with the wine industry have been undertaken numerous research and tested different technologies to exploit the products obtained at the winery.

As a culmination of these works is the technology developed by Research Institute for Viticulture and Winemaking "Magaraci", complex processing, in continuous stream of marc and wine yeast, obtaining alcohol, Ca tartrate, food oenocolorant, seeds grapes to produce oil, flour and fodder as a feed (Razuvaev). Following positive results by applying this technology in complex processing of the byporducts at the Wine Enterprise from Rostov, it was extended to over 20 units in Ukraine and Moldova.

At the Combinate combinat from Armavir, in the manufacture of fats and oils, it was organized on industrial scale the manufacture of oil from grape seeds.

Research Institute for Food Industry of Moldova has developed a technology to manufacture food oenocolorant, powder and concentrated form, by the method of travelling in countercurrent with andidrida sulfur solution.

Technological scheme for obtaining the concentrated pigment states to remove the impurities from the extract before merging.

To remove sugars, the steam without sulphite extract is fermented with yeast bread, filtered and purified using ion exchange resin.

Purified extract is condensed in the vacuum apparatus until content in dry matter of 40% turned to dust, with the following

composition:

- total extract % no less than.....30
- coloring substances g/l 50
- ash%, no more than 7
- pH of solution 3%..... 2.2–2.5

This pigment has been tested successfully in the manufacture of toffee, marmalade, soft drinks, pastries *etc*.

Also in Moldova it was asimilated the extraction technology of Ca tartrate from the distillation of grains, using ion exchange resins.

Experimental processing of wine yeasts in semiproduction conditions, performed at the oil mill and plant ethers Natasinsk and "Magaraci" wine factory demonstrated that, apart from alcohol, it can perform in the same distillation apparatus, the obtaining from precipitated yeast of cognac oil (ether).

It has been proposed and scientifically based the building of specialized factoryes in viticulture developed areas (Moldova, Crimea, Azerbaijan, Georgia), who will work throughout the year and will provide complex processing of products from the winery [SÎRGHI, 1992].

In California (USA) extraction of alcohol from the marc is mandatory, becouse for some types of fortified wines must be used only alcohol from wine.

To this end, Connie D. describes a continuous extractor for washing the nonfermented marc, manufactured by "Walley" company from Califormia.

With this installation, is extracted only the sugar from the fermented marc and only the alcohol from the fermented one. Tartaric compounds are not extracted.

Valorification of the se secundary products in the wine sector in our country

In our country, although the wine sector is well represented, Romania occupies the 7th place in the world acreage and 9th place for production, the secundary products of wine–making process do not know the variety of directions of valorification practiced in other countries with a developed viticulture and wine industry, as it was detailed in the previous chapter.

In the industrial sector belonging to both Vinalcool companies, as well as agricultural companies the wine yeast processing is done to recover the alcohol, used as yeast spirit. Processing of wine yeasts is done in industrial plants of the type cloyed or continue-column located in over 60% of counties, mostly grouped in the large wine pools.

Dregs of the yeasts left over from the distillation are discharged directly to sewer or landfill.

So far we do not have any data to prove the pulp processing for recovery of tartrate or other destinations, to one or another of the units in the vineries from our country.

Given the extremely valuable composition of these yeasts, we believe this unfortunate situation, both economically and also environmentally (this "waste", approximately 50 tons annually, with a heavy concentration of protein, vitamins, polluting waste water distillation units).

A further proof of the value of these wine yeasts is the offer of two Greek partners, to acquire substantial quantities of this by–product at a good price (half the price of wine). Acquisition is made in order to valorify on alcohol and organic acids.

The deal is a first for our country.

As for the marc, the product enjoys a special appreciation for other countries, here is rarely valued. We expressed such becouse 20–30 years ago it was processed to recover the alcohol either fresh or fermented, obtaining marc spirit or rectified alcohol.

We do not know why this practice was dropped, especially since today is the acute problem of profitability of enterprises and national economy needs require a high rentability.

In the grape processing industrial units, the marc is basically a quantitatively important waste, considering that 100kg of grape give around 10–18kg of marc.

The annual resulted amount is a problem for agriculture when it is not processed, requiring large storage areas, areas that are set aside due to increased acidity.

Marc disposal in the vineyard, as such, not composted, usually quite commonly used, is practically worthless, it is regarded as an extreme solution to solve this waste that the wine units must discard until the next

campaign.

There is information regarding the valorification of marc as feed, but it is sporadically and is not organized an industrial processing, which is much less understandable as it is known the acute need to feed livestock in our country.

In terms of grape seeds in our country there were not, until recently, concerns about the valorification of this important wine by–products.

The chemical composition of grape seed oil is mentioned only in C. Ille and G. C. Balanescu work of the Institute of Food Research (1963–1964), together with chemical composition and other seed oils.

Recent research has taken U. Iouras and R. Stanescu from Agronomy Institute Cluj–Napoca, following content in oil and fatty acid composition of seeds from several varieties of grapes grown in different geoclimatic conditions: Cluj Napoca, Simleul Silvaniei and Blaj.

Seeds' fat content between 8–20%, as the fatty acid composition of this oil, coresoponds to data provided by researchers from other countries; the only difference that was found in our larger amounts of linoleic acid (71–76%) and lower oleic acid (13.6%) than those determined in grape oil from other geographical areas (C18: 2 59–71% and C18: 1 15–25%).

Grape seed and oil extracted from them are subject to the research of a collective from ICCF.

At the Research Institute of Horti–vine V. Calugareasca, as well as in the Faculty of Food Chemistry–University of Galati, were developed and are ongoing researches on extraction of tannin from grape seeds.

All these concerns were left behind, at the experimental level.

In industrial wine units, grape seeds, in so significant amounts (about 30 tons/year) and content so valuable, remain included in the marc, at the state of waste.

Quantitative assessment of waste resulting from the wine grapes processing campaign

We consider useful the presentation of the quantitative elements (*Tables 1, 2*) to emphasize that these products are available in considerable quantities (about 100 000 tonnes), representing, as we have shown, a real ballast for agriculture and an aggressive attack to the environment.

Table 1.
Quantities of marc and yeast of Vinalcool Societies over the country.

County	Marc (tons)	Yeast (tons)
Alba	67	45
Arad	20	13
Arges	504	336
Bacau	408	272
Braila	270	180
Brasov	5	3,5
Buzau	2131	1421
Calarasi	90	60
Constanta	1377	919
Dâmbovita	36	24
Dolj	1028	686
Galati	823	548
Giurgiu	26	17
Ialomita	26	17
Iasi	3018	2012
Mures	69	46
Neamt	77	51
Prahova	1116	744
Satu Mare	3	2
Salaj	69	46
Sibiu	129	86
Teleorman	98	65
Timis	16	10
Tulcea	327	218
Vaslui	2175	1450
Vâlcea	31	21
Vrancea	5143	3429
Obor Bucur	163	109
TOTAL	19195	12797

From the *tables 1, 2* of the amount of waste (marc and yeast) it is clear that both in the SC Vinalcool, as well as the association, the counties with the highest weight in grape production hold the largest amounts of waste, too.The top 10 looks as follows: Vrancea, Constanta, Iasi, Prahova, Vaslui, Buzau, Galati, Tulcea, Alba, Mehedinti.

Total quantities of waste from winemaking campaign in 1995 are 65,852 tons of marc and 44,165 tons of yeast, therefore approximately 100,000 tons. It should be noted that the data presented are provided by the Department of Food Industry of the Ministry of Agriculture and refers to values recorded in the campaign for the wine of 1995, representing the current situation in

the wine industry. We could not make an assessment on a longer period of time, as production of wine and default products (marc and yeast), by SC (Ex Vinalcool sites) is about eight times lower after 1989 than the one before 1989.

The phenomenon is explained by the fact that after this date, there has been a recovery action of the areas planted with vines in favor of private producers, and on the other hand, because they were not legally obliged to surrender allowances of grapes to the state, respectively to Vinalcool units.

Table 2.

Quantities of marc and yeast by association, on counties

County	Marc (tons)	Yeast (tons)
Alba	1202	801
Arad	530	353
Arges	380	253
Bacau	458	305
Bistrita	116	77
Braila	498	332
Buzau	2264	1776
Calarasi	65	43
Cluj	42	28
Constanta	8551	5700
Dolj	550	366
Galati	2437	1625
Ialomita	489	326
Iasi	3965	2643
Mehedinti	1041	694
Mures	522	348
Olt	568	379
Prahova	4089	2726
Satu Mare	23	16
Sibiu	53	35
Timis	936	624
Tulcea	2344	1563
Vaslui	2641	1760
Vâlcea	407	271
Vrancea	12486	8324
TOTAL	46657	31368

A privileged situation have the agricultural societies (formerly IAS), which processes the yield realized on their own land; land whose surface has not undergone notable differences.

We also mention that in these data are not included the quantities of products made by the research institutes Horti-wine that solves this problem of the waste in their own way, initiating at the same time research on their valorification, as outlined in the previous chapter.

Conclusions

The secundary products of wine are: bunch, marc, seeds, yeast, tartaric deposits etc. These wastes are organic raw materials with chemical and biological composition that is extremely valuable: sugars, proteins, organic acids, oils, tannins, vitamins, pigments *etc*.

For extraction of various useful components, the oenological industry the secundary products are subjected to various processing techniques. By their processing is carried out additional high–value products and of urgent necessity, such as: alcohol, oil seeds, tannins, food pigments, tartrate, yeast feed, fertilizers, *etc*.

Summary of literature data revealed that, in general, countries with a developed wine industry valorify the resulted from the winery, making all secundary products useful.

Comparing the state of public opinion in this direction in our country, it is found that, on an industrial scale is performed only the processingof wine yeasts in order to recover the alcohol.

Concerns of other research institutes in order to obtain oil, tannins and proantocianilor from seeds of grape are only on experimental phase.

Quantitative Evaluation of secondary products in wine production, has highlighted the fact that they are available in considerable quantities (about 100,000 tons/year), at the same time representing a real ballast for agriculture and an environmental pollutant.

All this information demonstrates the opportunity and necessity of the proposed theme and requires research work in this direction.

Priority area to be addressed is the recovery of the pulp left after distilling yeasts by their mild hydrolysis and separation from the extract of organic useful components.

Research will be required for reevaluation of marc by turning a new approach, namely the valorification of the diffusion solution (sugar or alcoholic) as culture medium for yeasts fodder.

Another research direction is to exploit the marc washed and dried as an improver for certain categories of soil or as a substrate for different cultures.

This research will have, besides the

economical contribution an ecological contribution becouse for the moment the waste is discharged directly into sewers or landfills.

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