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FERMENTATIVE PROCESS FOR THE PRODUCTION OF GRAPE MARC ENRICHED YEAST - MICROPILOT LEVEL

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Abstract

Considering current food needs and environmental concerns worldwide, the full use of production and waste reduction is a practice that is increasingly present in our lives. Grape cultivation is one of the most widespread in the world, with more than 77 million tons of grapes harvested only in 2019. Main part of the production enters in the winemaking process. After the process completion, the main waste, grape pomace, is thrown in the field or, in the best case, used in composting processes, losing compounds with significant value. The aim of this paper was to obtain products based on yeast enrich with grape pomace as winemaking by-product (Feteasca Neagră variety), rich in polyphenols, and with high antioxidant activity. The grape pomace used for fermentations was obtained following the winemaking processes carried out at the Didactic Research and Development for Viticulture and Pomiculture Pietroasa - Istrița, resort with a history of over 120 years in viticulture. The product thus obtained needs several more tests for the correct evaluation of its superior characteristics in terms of antioxidant properties.

Key words*: grape marc, yeast, fermentations, micro pilot, Pietroasa, Fetească Neagră.*

INTRODUCTION

Globally, grape cultivation has impressive values every year, reaching 73.5 mil. tons in 2017, going up to 80 mil. tons in 2018 and keeping almost the same level, 77.1 mil. tons, in 2019, values that make it one of the most widespread crops worldwide (FAO, 2020).

With current food needs and environmental concerns worldwide, the full use of production and waste reduction is a practice that is increasingly present in our lives.

Pomace is the main residue (a solid material) generated from the pressing of grape. (Munekata et al., 2021). Grapes are one of the most valued crops in the world. The average annual production worldwide exceeds 60 thousand tons. Around 80% of grapes are used for winemaking. (Ferreira and Santos, 2022). Grape pomace is the main residue associated with the antioxidant activity because of the polyphenols content and tannins.

It is well known that fermentation in a solid state is performed for obtaining potential feed additives for animal production. In the case of broiler chicks, the incorporation of fermented grape pomace in animal diets produced heavier animals with increased serum levels of catalase (a component of the antioxidant defense system) (Fang, J.; Cao et al., 2015). Grape pomace represents approximately 10-30 % of the mass of crushed grapes and contains unfermented sugars, alcohol, polyphenols, tannins, pigments, and other valuable products. (Muhlack et al., 2018). Due to the economic and environmental interest, this study aimed to demonstrate that grape pomace can be used as a fermentation substrate due to its high polyphenol content and antioxidant activity, adding value to the production of yeast biomass.

MATERIALS AND METHODS

For the fermentation processes, a yeast strain belonging to the Saccharomyces cerevisiae species was used, previously isolated and identified from Feteasca Neagră grapes by specific methods described by Dumitrache et al., 2020.

Biological material

The grape pomace used for fermentations was obtained following the winemaking processes carried out at the Didactic Research and Development for Viticulture and Pomiculture Pietroasa - Istrița, resort with a history of over 120 years in viticulture (Dejeu, 2013).

In the research conducted by Borges and collab. (2020) described that grape pomace drying is considered to be an essential process for grape pomace conservation and stabilization because is susceptible to microbial degradation due to its high moisture content. According to this, the grape pomace of Fetească Neagră variety was dehydrated at 45°C and then ground with a laboratory mill to a granulation that allowed the fermentation in the bioreactor without any problems.

Fermentation at the micro-pilot level goes through the following stages, i.e: (*i*) obtaining static culture (pre-inoculum); (*ii*) obtaining the liquid inoculum; (*iii*) micro-pilot fermentation; (*iv*) post-fermentative processing.

Pre-inoculum preparation

Pre-inoculum was obtained by seeding a maintenance culture (YPG - agarized) on - tubes and incubating for 48 hours at 30°C. After the incubation it was analysed macroscopically to verify its viability and microscopically to determine the degree of development and its purity.

Inoculum preparation

The liquid inoculum was made by seeding the pre-inoculum culture in sterile liquid culture medium based on sucrose, yeast extract and peptone. Each raw material for the culture medium was weighed and dissolved in 400 mL of distilled water, evenly distributed in 4 Erlenmeyer flasks and sterilized at 121°C for 20 minutes. Then, each Erlenmeyer flasks with sterilized medium it was seeded with 2 pre-inoculum inclined tubes. The inoculum thus obtained was incubated for 20-22 hours at a temperature between 28-30°C and at a stirring rate between 170-240 rpm (Figure 1).

The obtained inoculum culture was analysed microscopically to determine its purity and the degree of budding of the yeasts (development stage) (Figure 2).

Figure 1. Aspects of yeast inoculum preparation by shacking in the incubator

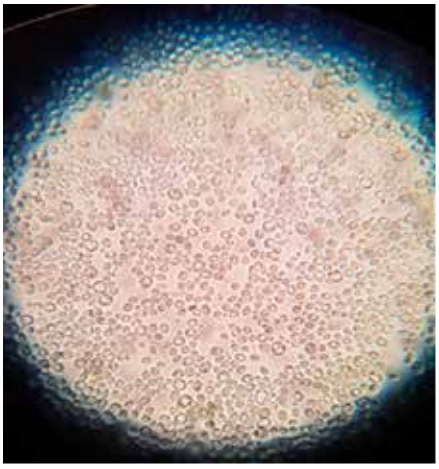

Figure 2. Microscopic examination of the purity and budding of the yeast inoculum

Micro-pilot fermentation

For the micro-pilot level, the fermentation processes were performed in single batch system Biostat B PLUS micro fermenter (6 L total volume), at a working volume of 4 - 4.5 L. For micro-pilot fermentations, the used culture medium consisted of: yeast extract, $NH_4H_2PO_4$, KCl, $MgSO_4$, H_2O and sugar. These nutrients have been added to the bioreactor vessel and sterilized with it and its accessories at 121°C for 20 min. After completion of the sterilization process, the vessel with sterile medium was allowed to reach room temperature.

Before inoculating the culture medium with the previously obtained liquid inoculum, the apparatus was brought to the working parameters, respectively temperature of 30°C; stirring with a speed between 250-350 rpm; pH between 4-5.

The inoculation rate used for fermentation was 10-15% (Bărbulescu et al., 2021).
To keep a continuous fermentation, during the process, a sterile solution of 40 % sucrose was added depending on the evolution of the fermentations. To determine the favourable time for the addition of sucrose, samples were taken and the total sugar content was determined using a manual refractometer, model Kruss Handheld Refractometer HR10 (Figure 3).

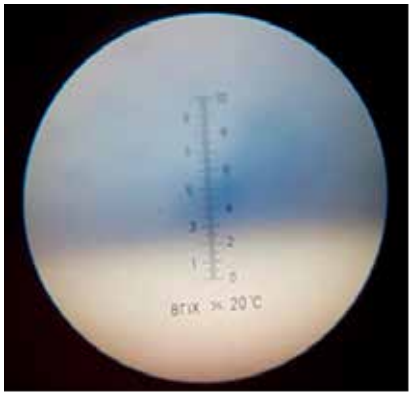

Figure 3. Aspects of the total sugar content reading on Kruss Handheld Refractometer HR10

When the situation required, one or two drops of anti-foam was added to break the foam and prevent entering in the device filters.
During fermentation the pH was maintained at a value between 4 - 5 with ammonia solution.
The grape pomace was added at different stages of fermentation and in different quantities (Figure 4).

Figure 4. Fermentation after 12 hours of cultivation with grape pomace added

Based on some previous tests at laboratory level, 2 methods of adding grape pomace were used: (*i*) a single addition of dry pomace (Table 1) and (*ii*) gradual addition of dry pomace, in several smaller doses (Tables 2 and 3). The total addition of grape pomace for a 4 L working volume of bioreactor was 50 g.

Table 1. Batch Fermentation with a single dose of 50 g of added dry grape pomace

Time of cultivation (h)	pH	Dry matter (%)	Remarks
0	4.8	10.5	
4	4.73	9	
6	4.73	7.5	
8	4.73	5.5	Addition of 50 g dry Fetească Neagră grape pomace
9	4.74	5	
10	4.74	4.5	
11	4.74	4	
12	4.75	3.5	Addition of sterile solution of sucrose
13	4.74	5.5	
14	4.74	5	
15	4.74	4	
17	4.74	3.5	Addition of sterile solution of sucrose
18	4.74	4.5	
19	4.75	4.5	STOP fermentation

Table 2. Batch Fermentation with two doses of 25 g of added dry grape pomace

Time of cultivation (h)	pH	Dry matter (%)	Remarks
0	5.3	10	
4	4.2	6	
6	4.74	4.5	
8	4.74	3.5	Addition of dry Fetească Neagră grape pomace - 25 g;
9	4.74	3	Addition of sterile solution of sucrose
10	4.75	4	Addition of dry Fetească Neagră grape pomace - 25 g;
11	4.75	3	Addition of sterile solution of sucrose
12	4.74	3.8	
13	4.74	3.5	
14	4.74	3	Addition of sterile solution of sucrose
15	4.74	4.5	
17	4.74	4	
18	4.74	3.5	
19	4.74	3.5	STOP fermentation

Table 3. Batch Fermentation with three doses of 16.7 g of added dry grape pomace

Time of cultivation (h)	*pH*	Dry matter (%)	Remarks
0	4.8	10	
4	4.74	8.5	
6	4.74	6.5	
8	4.74	4.5	Addition of dry Fetească Neagră grape pomace - 16.7 g
9	4.74	4	
10	4.74	3	Addition of dry Fetească Neagră grape pomace - 16.7 g
11	4.74	5	Addition of sterile solution of sucrose
12	4.73	4	Addition of dry Fetească Neagră grape pomace - 16.7 g
13	4.74	3.5	Addition of sterile solution of sucrose
14	4.74	5	
15	4.73	3.5	Addition of sterile solution of sucrose
17	4.74	4	
18	4.74	3.5	
19	4.74	3.5	STOP fermentation

Post-fermentative processing

After completion of the fermentation, the fermented medium was left to decant in the cold for 24 h, after which it was subjected to a mechanical separation by centrifugation to obtain the final product: a yeast cream with grape pomace (Figure 5).

Figure 5. Product obtained: yeast cream with added grape pomace

To purify the product, successive washes were performed with sterile distilled water at 3500-4500 rpm, for 5 - 10 minutes at each wash.

The wet cells weight (g/L) was measured after the post-fermentation process at the end of the cultivations (Bărbulescu et al., 2010).

Determination of total polyphenols content (TPC) and antioxidant activity.

The determination of the polyphenols content and the antioxidant activity was performed using the infrastructure from the Research Center for the Study of the Quality of Agri-Food Products within the University of Agronomic Sciences and Veterinary Medicine from Bucharest.

In order to determine the total concentration of polyphenols (TPC), the Folin - Ciocâlteu method used by Stan et al., in 2020, and in terms of antioxidant capacity, the DPPH method (2.2-diphenyl-1-picrylhydrazyl) was used, with subsequent modifications made by Ion et al. in 2020.

RESULTS AND DISCUSSIONS

In order to obtain the final product, previous laboratory experiences were used regarding the isolation, identification of the yeasts, as well as the optimization of the fermentative processes to yeast fermentation capacity (Dumitrache et al., 2020).

Following the experimental batches performed, it can be seen from Figures 6, 7 and Table 4 that the addition in 3 portions of 16.6 g of dry grape pomace to 4L working volume gave the best results, reaching a total of 77 g/L wet cell weight (WCW).

Table 4. WCW results

Batch	**WCW** g / L
Fermentation with a single dose of 50 g of added dry grape pomace	67.75
Fermentation with two doses of 25 g of added dry grape pomace	62.5
Fermentation with three doses of 16.7 g of added dry grape pomace	77

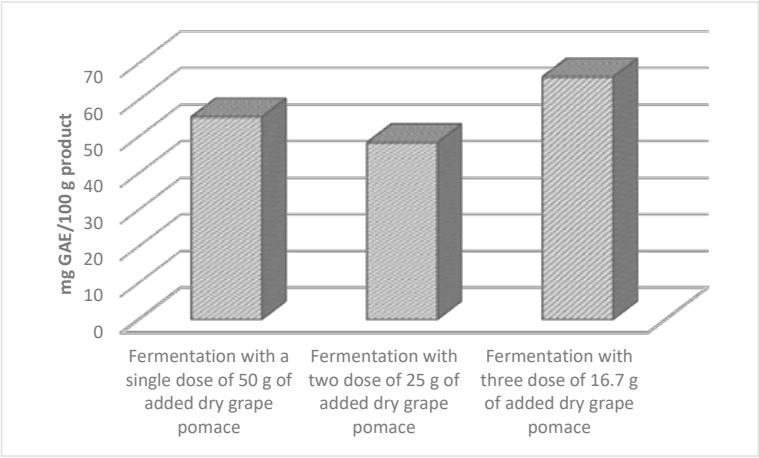

Figure 6. Total phenolic content of different samples of fermented dry grape pomace

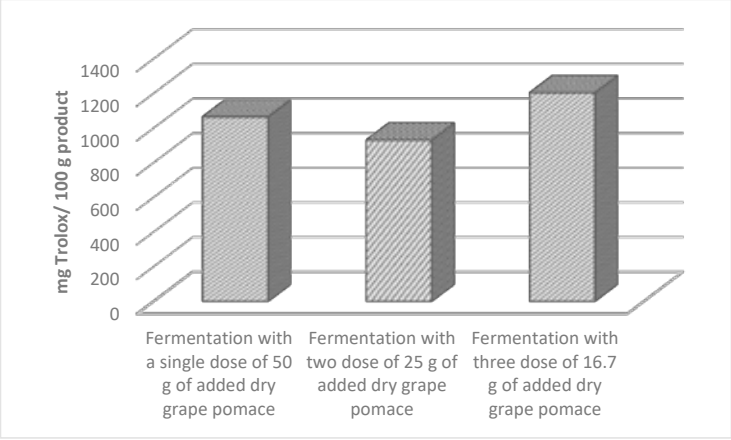

Figure 7. Antioxidant activity of different samples of fermented dry grape pomace

It is observed that the addition of the sugar source together with three portions of dried pomace positively influenced the fermentation improving the cellular concentration.

After the post-fermentation process was obtained 305 g/4 L (77 g/L WCW - wet cell biomass) compared with the addition of the dried pomace in two portions, when the amount of biomass was 250 g/4 L (62.5 g/L - WCW) and respectively in a single portion (271 g/4 L (67.75 g/L WCW). More specifically, fermentation with addition of three dried pomace portions had an increase of 12.78% WCW compared to fermentation with addition of one dried pomace portions, and 20.78% WCW compared to fermentation with additions of two dried pomace portions.

The inhibitory effect was observed in the second experiment (with the addition of pomace in two portions), respectively in the first experiment (with the addition of a single dose of pomace) by the growth rate of the fermentation.

The yield of biomass substrate is positively influenced by the way of addition of grape pomace as substrate source of fermentation.

For the *Saccharomyces cerevisiae,* strain PM1 isolated from Fetească Neagră grape variety and identified by RFLP analysis of 5.8S-ITS region by Dumitrache et al., 2020 were also pointed their capacity for polyphenols biosorption and the higher antioxidant capacity. The different behavior related to biosorption of polyphenols depends on the amount of addition of dried grape pomace and also by the time of cultivation.

The study performed by Rajha et al. (2014) proved that the accelerated solvent extraction (ASE) of phenolic compounds from wet and dried grape pomace, at 45°C, was conducted and the highest phenolic compounds yield (PCY) for wet (16.2 g GAE/100 g DM) and dry (7.28 g GAE/100 g DM) grape pomace extracts were obtained with 70% ethanol/water solvent at 140°C. Compared with this study, our content from our final product, yeast biomass enriched with grape pomace, show a higher content in polyphenols 66.3 mg/100 g dry pomace. In the research study performed by Negro et al. (2021) they investigated four grape pomace which shown different polyphenols and antioxidant activities. In our study content of polyphenols is corelated with antioxidant activity.

CONCLUSIONS

During the trial fermentations performed on laboratory level scale (working volume of 4-4.5 L) can be observed that the gradual addition of dried pomace had a positive effect on the final amount of wet yeast biomass enriched with grape pomace respectively polyphenols.

In the fermentations with a single addition of dry grape pomace, a slowdown of the growth phase is observed immediately after the addition (visible from the slowdown in carbon consumption), which may be due to the fact that the yeast culture needs a period of adaptation to new cultivation conditions. In fermentations with gradual addition of dried pomace, in three portions, no negative effect of the growth phase was observed.

The time of addition was established to be in full process of yeast growth - exponential phase (in the interval 12 hours from the start of fermentation), with a pH range between 3 and 4.5, pH range in which the grape pomace is located.

The methodology for adding the source of dry grape pomace during fermentation was established. The optimal variant for the development of biomass and for obtaining a high concentration of polyphenols and antioxidant activity was in three additional portions (16.6 g dry pomace/4 L working volume with fermentation medium). This interval was chosen so as not to make grape pomace an intruder in fermentation, but to be easily accepted. After a short period of accommodation of the fermentation, carbon source was added. For the fermentation with three additional portions, the grape pomace and carbon additions alternated, keeping between them a shorter period of accommodation than in the case of the other two batch.

The aim in the future is to evaluate different yeast biomass based on total polyphenol content in order to obtain valuable bio-based ingredient with potential to be used as nutritional supplements.

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