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Enzymatic pre-treatment of grape seeds for an oil with higher antioxidant activity

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SUMMARY: The paper investigates the effect of the enzymatic pre-treatment of grape seeds from six Romanian cultivars on the oil extracted. The grape seeds of some white and red Romanian grape varieties were separated from winery waste, washed, dried and ground, with the oil then obtained by extraction with petroleum ether. The extraction was performed directly or after a preliminary treatment with a commercial *pectin lyase*. The enzymatic procedure applied was more cost effective compared to other treatments previously described in which a cocktail of enzymes was used. The quantity of the extracted oil was measured in both types of processing, with an increase being observed for pre-treated samples. The fatty acid profiles (FAPs) of the oils resulted for the treated and untreated seeds were determined. No change in the composition was noticed. The reductive power of these oils was also investigated. Compared to the untreated samples for the same variety, the enzyme pre-treatment resulted in a superior antioxidant capacity.

KEYWORDS: *Antioxidants content; Fatty acid profile; Grape seeds; Pectin lyase treatment; Statistical analysis*

RESUMEN: ***Pretratamiento enzimático de semillas de uva para un aceite con alta actividad antioxidante***. En este artículo se investigó el efecto del tratamiento enzimático de semillas de uva de algunos cultivares rumanos sobre el aceite extraído. Las semillas de uva de variedades seleccionadas de uva rumana blanca y roja se separaron de los residuos de la bodega, se lavaron, secaron y molieron, a continuación el aceite se obtuvo mediante extracción con éter de petróleo. La extracción se realizó directamente o después de un tratamiento preliminar con una liasa de pectina comercial. El procedimiento enzimático aplicado es más rentable en comparación con otros tratamientos descritos anteriormente en los que se utilizó un cóctel de enzimas. La cantidad de aceite extraído se midió en ambos tipos de procesamiento y se observó un aumento para las muestras pretratadas. Se determinaron los perfiles de ácidos grasos (PAG) de los aceites resultantes de las semillas tratadas y no tratadas. No se notó ningún cambio en la composición. También se investigó el poder reductor de estos aceites. En comparación con las muestras no tratadas para la misma variedad, el pre-tratamiento enzimático dio lugar a una capacidad antioxidante superior.

PALABRAS CLAVE: *Análisis estadístico; Contenido antioxidante; Pectina liasa tratamiento; Perfiles de ácidos grasos*

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1. INTRODUCTION

The evaluation of any economic activity may be performed based on the economic value added (EVA) model developed in 1982 by G.B. Stewart, which is still used successfully (Stewart, 2013). In such context the valorization of the biomass waste from the industrial production of wine is important, as obtaining added value is a priority for any sustainable economy.

Romania is a known wine producer, being the 11th producer in the world and the 5th in Europe (European Commission, 2019). A sustainable development for winery implies the adequate management of resources in the production chain and reduction of waste (Maicas and Mateo, 2020). Waste minimization through technological innovation will generate an EVA equity indicator (Machová and Vrbka, 2018). A waste reduction policy is recommended for achieving a sustainable wine making process (Devesa-Rey *et al.*, 2011) and the implementation of the "zero waste" concept (Donner *et al*., 2020).

Winery wastes are an important part of wine production. Grape pomace is a waste which results as a by-product from the must production; it is composed of around 30% stems, 30% seeds and 40% skins and pulp**.** It is considered an agro-industrial waste, representing about 25% (w/w) of the weight of grapes processed and more than 9 million tons annually (Sirohia *et al.*, 2020, Cvejic Hogervorst *et al.*, 2017).

There are several proposals for solving the problem of pomace. It can be used in different applications such as functional foods and supplements, pharmaceutical and cosmetic products (Galanakis, 2020). The dispersal of it into landfill seems ecologically inappropriate (Dwyer *et al.*, 2014). Viable solutions for winery waste valorization are: oil production (Al-Juhaimi and Ozcan, 2018), manufacture of composite materials (Barbieri *et al.*, 2013), extraction of the antioxidants for food supplements (Nowshehri *et al.*, 2015), bioconversion to valuable chemicals or biofuels (Rani *et al.,* 2020; Zacharof, 2017).

A better biomass valorization implies the separation of the pomace components (Toscano *et al.*, 2013). The seeds represent a great part of these wastes, approximately 47% on dry base (Zhang *et al.*, 2017), making their valorization vital.

The paper investigates the valorization of grape seeds by oil extraction. Residual grape seeds from six Romanian cultivars were processed. In order to increase the outcome, a pre-treatment of the seeds with a commercial *pectin lyase* was experimented. The enzymatic treatment seems appropriate due to the progress in the industrial production of enzymes as well as their numerous industrial applications derived from their properties, namely: low toxicity, energy saving due to mild work conditions, biodegradability, etc. (Choi *et al.*, 2015). An improvement in oil quantity and/or quality was predicted.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Sample preparation

The grape seeds were isolated from the pomace resulting from the wine making process, washed, dried for 24 hours (h) at room temperature, and ground. Seeds from the grape varieties of the 2015 harvest were investigated, namely four red brands, including Cabernet Sauvignon, Feteasca Neagra, Merlot, Pinot Noir and two white brands Columna and Riesling Italian. The material was supplied by the winery of Murfatlar, situated in Dobrogea, a south-eastern region of Romania.

2.1.2. Commercial enzyme

The pectin lyase solution (Pectinex XXL, activity 10.000 U mL^{-1} according to the supplier) was purchased from Novozymes A/S (Bagsvaerd, Denmark).

2.1.3. Chemicals

The 10-14% BF_3 solution in methanol was supplied by Merck (Darmstadt, Germany). Petroleum ether (b.p. 40-60 ºC), analytical grade methylene chloride, methanol, and 96% ethanol solvents were purchased from Sigma Aldrich and were used as delivered. The citric acid and disodium phosphate dihydrate for the buffer solution were supplied by Sigma Aldrich.

2.2. Equipment and procedures

UV-Vis spectra (200-800 nm) were acquired on a Helios Beta apparatus with Vision software (Thermo Electron Corporation, Waltham, MA, United States).

The ^{1}H-NMR spectra were obtained on a Bruker Avance III 400 MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany).

The GC analyses were performed on an Agilent Technologies 7890A instrument (2850 Centerville Road Wilmington, DE 19808-1610 USA), provided with a flame ionization detector.

2.2.1. Enzymatic treatment

Portions of 10 g ground grape seeds were kept for certain amounts of time in a solution of 3 mL commercial enzyme and 37 mL buffer solution of pH 3.5 (0.1M citric acid and 0.2M Na_2HPO_4, 2.3/1 volume ratio), in 100 mL capped glass amber flasks, at room temperature (20 ºC), with gentle intermittent stirring. The optimal time for treatment was established based on the oil yield (see Figure 1). For each grape seed variety the experiments were conducted in triplicate. After treatment, the seeds were separated by filtration, washed and dried before extraction.

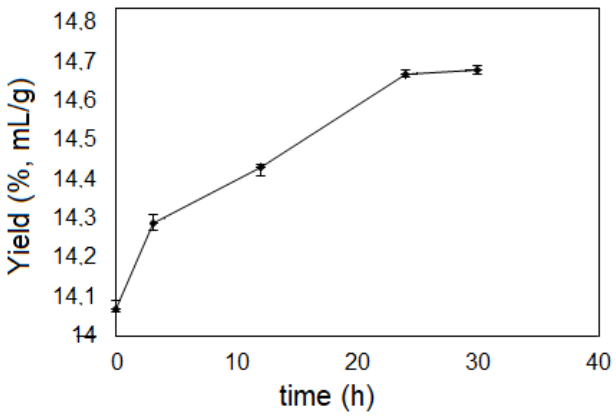

Figure 1. Variation in the extracted oil (mL oil/100 g seeds) with the pre-treatment time for the Pinot Noir variety (Standard deviation for n = 3)

The residual solution was concentrated in a Heidolph rotary evaporator, in a water bath (50 ºC), and the residue was studied by NMR spectroscopy.

For checking the buffer effect on the grape seed a number of experiments were performed by keeping the seeds in the buffer solution (ratio w/v = 1/4) for 24 hours, at room temperature. The quantity of the oil from these samples was in the same range as the corresponding untreated oil.

2.2.2. Oil extraction

The oil samples were obtained from the dried ground seeds (pre-treated or not with enzyme) by the Soxhlet extraction method with petroleum ether (b.p. 40-60 ºC), following the protocol ISO 659 (2009). The solvent was partially recovered by evaporation under atmospheric pressure, heated on a water bath at 65 ºC, using a Heidolph rotary evaporator. The weight of the oil was measured with an analytical balance (accuracy of ± 0.0001 g) and the corresponding volume was calculated using the density value for grape seed oil of 0.92 g/mL (Ceriani *et al.*, 2008). The value of the density was confirmed by checking on a number of experimental samples. The experiments were performed in triplicate, the average volumes of oil being presented in Figure 2. The initial quantity of seeds was the same for both types of experiments (with or without pre-treatment) as measured before pre-treatment.

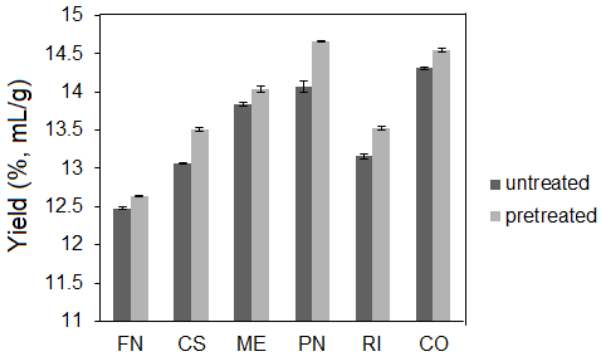

Figure 2. The oil amount (mL/100 g seeds) obtained by extraction from untreated and enzyme pre-treated seeds. (Standard deviation for n = 3; FN – Feteasca Neagra, CS - Cabernet Sauvignon, ME - Merlot, PN - Pinot Noir, RI - Riesling Italian, CO – Columna)

2.2.3. GC analysis of the grape seed oil composition (FAP)

The grape seed oil FAPs were determined following the ISO 12966 (2015) protocol. The free fatty acids were obtained by treatment of 0.5 mg oil with 0.9 mL NaOH 0.5 M solution in methanol, at reflux, for 10 min. Then, they were esterified by treatment with 0.1 mL BF_3 solution and 0.8 mL methanol, at reflux, for another 10 min. After dilution with 4 mL distilled water, the fatty acid methyl esters (FAMEs) were extracted with methylene chloride and were submitted to a standard gas chromatographic (GC) analysis. GC analysis was performed on a highly polar capillary column Supelco SP™ 2560 (100 m × 0.25 mm, 0.20 µm film) with a biscyanopropyl stationary phase. A certified reference material (Supelco™ 37 Component FAME Mix) was used for the identification of the FAPs

(see table 1). For each oil sample, three GC analyses were performed. The composition was expressed as a percentage based on the ratio between each peak area and the total area.

2.2.4. Determination of the reductive power of the oil samples

The CUPRAC assay (Apak *et al.*, 2008) was applied to assess the total antioxidative capacity (TAC) of the oil. Samples of oil extracted by sonication in ethanol (1/10, v/v) were treated with 1 mL $CuSO_4$ 10^{-2} M aqueous solution, 1 mL of 7.5 × 10^{-3} M Neocuproine ethanolic solution, 1 mL CH_3COONH_4 buffer (pH 7) and 0.4 mL distilled water. After stirring for 30 min, at room temperature, the absorbance (Abs) at 450 nm was measured. TAC was expressed as a trolox equivalent (TE, μmol per 100 g oil) based on a previously drawn calibration curve ($y = 0.058x$; $R^2 = 0.99$). The average values obtained from three experiments for each oil sample are presented in Table 1.

2.2.5. Determination of the total polyphenol content (TPPC) of the grape seeds

The polyphenols (PPs) were quantified using the known Folin-Ciocalteu (FC) method (Ballus *et al.*, 2015). Dried seeds (2 g), before and after the oil extraction, were extracted with 9 mL ethanol/water (2/1, v/v). Extract samples (0.1 mL) were mixed with the FC reagent (0.5 mL), distilled water (1.5 mL) and, after 5 min, with 20% Na_2CO_3 solution (1.5 mL). The mixture was incubated for 2 h, at room temperature, in the dark. The Abs at 750 nm was measured vs. a blank. The analyses were performed in triplicate, for seeds with or without enzymatic pre-treatment, before and after oil extraction. The TPPC was expressed as gallic acid equivalents (GAE, mg per 100 g dried seeds) derived from a previously obtained calibration curve ($y = 0.061x$; $R^2 = 0.98$) (Table 2).

2.3. Statistical analysis

To identify sample clusters the principal component analysis (PCA) was applied to the grape seed oil compositions. The independent variables were selected by PCA. The ANOVA program was used for the comparison of the mean values. Data analysis was performed using the XLSTAT 2015 software (Addinsoft).

3. RESULTS AND DISCUSSION

3.1. Effects of the pre-treatment with pectin lyase on the grape seed oil yields

The research was focused on the valorization of grape seeds from the waste of a Romanian winery

Table 1. FAPs and antioxidant content in the extracted grape seed oil

Cultivar	FN	FNE	CS	CSE	ME	MEE	PN	PNE	RI	RIE	CO	COE
Fatty acid	**FA Content (%)**											
Palmitic and Stearic	10.58±0.02	10.71±0.01	11.11±0.02	11.29±0.01	9.02±0.02	9.55±0.03	10.34±0.01	10.42±0.01	9.88±0.02	9.99±0.03	8.78±0.01	8.83±0.02
Oleic (18:1 cis-9)	15.47±0.01	15.84±0.03	12.14±0.02	12.36±0.03	12.18±0.02	12.62±0.02	14.39±0.01	14.53±0.03	18.10±0.01	18.18±0.01	17.85±0.01	17.90±0.01
Linoleic (18:2 cis,cis-9,12)	73.76±0.02	73.28±0.01	76.48±0.02	76.12±0.01	78.34±0.01	77.60±0.01	75.05±0.02	74.76±0.01	71.79±0.02	71.54±0.03	73.13±0.01	73.00± 0.02
Linolenic (18:3 cis,cis,cis 9,12,15)	0.19±0.01	0.22±0.01	0.27±0.01	0.22±0.02	0.28±0.02	0.24±0.01	0.23±0.01	0.27±0.01	0.23±0.01	0.28±0.01	0.24±0.01	0.27±0.01
TAC (μmol TE /100 g oil)	72.84±1.14	176.75±1.31	62.43±1.49	131.64±1.45	109.26±1.32	216.18±1.19	134.19±1.28	234.48±1.54	33.45±1.31	58.73±1.40	50.14±1.33	85.12±1.49

Standard deviation for n = 3

FAPs - fatty acid profiles, FA - fatty acids, TAC - total antioxidative capacity, TE - trolox equivalent, FN – Feteasca Neagra, FNE – Feteasca Neagra pre-treated with enzyme, CS - Cabernet Sauvignon, CSE - Cabernet Sauvignon pre-treated with enzyme, ME -Merlot, MEE –Merlot pre-treated with enzyme, PN - Pinot Noir, PNE - Pinot Noir pre-treated with enzyme, CO - Columna, COE - Columna pre-treated with enzyme, RI - Riesling Italian, RIE - Riesling Italian pre-treated with enzyme.

Table 2. Polyphenol content in grape seeds

Sample Variety	PN	ME	FN	CS	CO	RI
Analyzed sample	**Polyphenols as *GAE* (mg/100 g seeds)**					
Initial	132.27±1.33	148.52±1.25	133.74±1.33	149.26±1.35	133.74±1.39	134.48±1.34
After extraction	127.09±1.53	128.57±1.34	130.05±1.44	144.09±1.59	128.57±1.35	127.09±1.33
After pre-treatment and extraction	124.14±1.49	125.62±1.43	115.27±1.48	135.22±1.54	127.83±1.58	124.14±1.34

Standard deviation for n = 3
GAE - gallic acid equivalents, PN - Pinot Noir, ME -Merlot, FN – Feteasca Neagra,
CS - Cabernet Sauvignon, CO - Columna, RI - Riesling Italian.

Murfatlar starting with the production of the grape oil. The oil obtained from grape seeds is a valuable material (Shinagawa *et al.*, 2015). Recent clinical trials showed the beneficial effects of this oil on human health (Kaseb and Biregani 2016; Ismail *et al.*, 2016).

The solvent extraction standardized method used for the oil separation is simple and less energy demanding than other procedures (Castro-Lopez *et al.*, 2016). The yield is relatively high due to the permanent contact of ground seeds with clean solvent, which favors the extraction. The grinding of the seeds helps due to the generation of a larger contact area for seed-solvent. The solvent used, petroleum ether, is industrially accessible and not so toxic because of degrading rapidly in soil and water and having a half-life of 3-8 days in the air (Nalliah, 2014).

Vegetable oils may be produced also by cold pressing the seeds but the yield is usually lower and it is difficult to obtain a constant quality of the product (Ustun–Argon *et al.,* 2020). The yield may be improved by using shockwaves but the process is complex and not always economically viable (Marousek, 2015a).

By comparison with other methods the solvent extraction of the grape seed oil has a low processing cost and is easy to handle (Galankis, 2020).

For improving the extraction process of bioactive compounds a number of procedures have been experimented, among which can be found supercritical fluid extraction, pressurized liquid extraction, microwave and ultrasound assisted extraction, etc. (Azmir *et al.*, 2013; Kumar *et al.*, 2017). Some of these procedures require investments which are suitable only for a large scale production.

Improvements of the extraction process may be done by pre-treatment of seeds before extraction. The literature has reported the following procedures: heating (Ustun–Argon *et al.,* 2020), shockwave treatment (Marousek, 2015a), enzymatic hydrolysis (Passos *et al.*, 2009) or a combination of these methods (Marousek *et al.,* 2015b).

According to the literature (González-Centeno *et al.*, 2010), pectins are polymer constituents of the cell walls in fresh grapes or in grape pomace, which bind other constituents. The study of oil distribution inside the seed showed its presence near the external tegument (Pope *et al.*, 1993). There is a relatively strong interaction between the oil and the seed walls leading to supramolecular structures (Scollary *et al.*, 2012) which block oil removal. Thus, to improve oil extraction, an enzymatic pre-treatment was carried out, with an enzyme specific for pectin breaking. The enzymatic treatment (see paragraph 2.2.1) was performed in batch mode, by maintaining the seeds in a buffered solution of Pectinex XXL, at room temperature and pH 3.5, parameters recommended for this type of enzymes (Najafian *et al.*, 2009). The *pectin lyase* cleaves the pectin (Yadav *et al.*, 2009) giving water soluble compounds (saturated and unsaturated pectic-oligosaccharides). The fragmentation process is improved by the presence of citric acid, which acts both as buffer and ligand for calcium ions (Stanescu *et al.*, 2010). Thus, these ions, which reinforce the pectin structure by making bridge bonds between chains (Ochoa-Villarreal *et al.*, 2012), are removed, aiding in the elimination of pectins. The fracture of the cell walls facilitates the access of solvent and improves the oil extraction yield. The chosen pH was the optimal one for the commercial enzyme used. Room temperature was suitable for the enzyme and did not require added cost for heating. The only parameter to be established was the treatment time. An optimal time of 24 hours was established for the enzymatic treatment based on the volume of the resulted oils for different time ranges (see Figure 1).

The enzymatic pre-treatment of seeds, for 24 h with *Pectinex XXL*, led to more oil, the quantities

obtained being 101.3-104.3% compared to untreated seeds (see Figure 2). Comparable results for the enzymatic pre-treatment of seeds have been found by other researchers (Passos *et al.*, 2009). The performance of the described procedure consisted of lower additional costs than those with enzyme cocktails (Passos *et al.*, 2009; Marousek *et al.,* 2015). A preliminary calculation (Tociu, 2019a) indicated a reduced cost (of over 100 times) for the pre-treatment with only Pectinex XXL compared to the cost of the treatment with a mixture of *cellulase*, *xylanase* and *pectinase* performed by Passos *et al.,* (2009), the differences in oil yield being insignificant.

Unfortunately, there are limitations to the application of enzymatic treatments such as the reproducibility of enzyme biosynthesis and the possible negative effects of the stabilizers on commercial products. These are impediments to the application of enzymatic treatments at large scale in processes where the enzyme properties (content, activity) are of great importance.

3.2. Effects of pre-treatment with pectin lyase on the oil properties

3.2.1. FAPs of the grape seed oils

One of the most important features of lipids is their fatty acid profile (FAP). The previous research involving enzymatic pre-treatment (Passos *et al.*, 2009; Marousek *et al.,* 2015b) did not check this aspect for the oil applications. Thus, the extracted oils were analyzed by a GC standard method (see paragraph 2.2.3). The FAPs of the oil samples are presented in Table 1.

The main component of these oils was linoleic acid, a representative polyunsaturated fatty acid (PUFA). The saturated fatty acid (SFA) content was around 10%. Similar FAPs were revealed for Portuguese grape varieties (Fernandes *et al.*, 2013) and Spanish wines with protected denomination of origin (Bada *et al*., 2015). Due to their low SFAs and high linoleic acid contents these oils present good nutritional qualities (Alsharari *et al.*, 2017). According to the experimental results the pre-treatment changed the FAPs only slightly (≤ 0.4%).

As expected, the statistical analysis revealed a close correlation between PUFA and linoleic acid ($y = 1.004x+0.1003$; $R^2 = 0.99$). The grape variety showed an impact on the content in fatty acids in oil. Principal

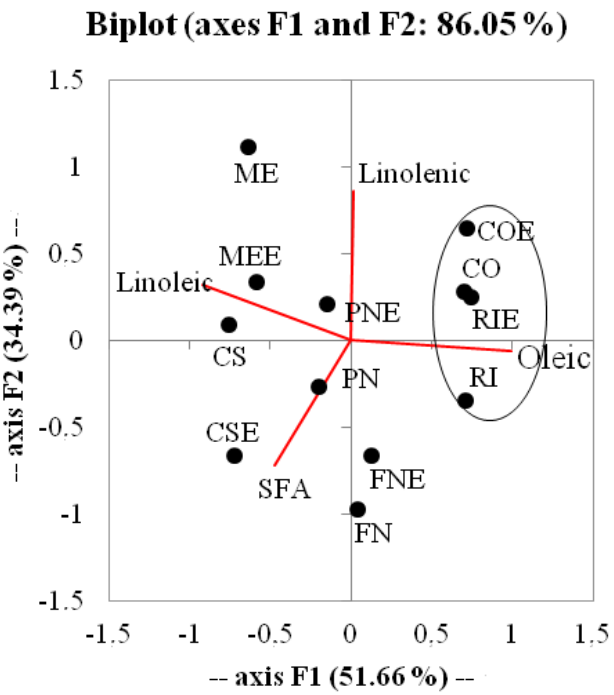

Figure 3. Principal component analysis based on FAPs. (CS - Cabernet Sauvignon, FN – Feteasca Neagra, ME -Merlot, PN - Pinot Noir, CO - Columna, RI - Riesling Italian, CSE - Cabernet Sauvignon pre-treated with enzyme, FNE – Feteasca Neagra pre-treated with enzyme, MEE -Merlot pre-treated with enzyme, PNE - Pinot Noir pre-treated with enzyme, COE - Columna pre-treated with enzyme, RIE - Riesling Italian pre-treated with enzyme, SFA - saturated fatty acids)

component analysis (PCA) applied to FAPs disclosed the differences or similarities of the analyzed oils (Figure 3). The PCA analysis revealed the following eigenvalues: F1: 2.067, F2: 1.375, F3: 0.558, F4: 0.000. Thus, the variance values of the principal components (PCs) were 51.66% (F1), 34.39% (F2), 13.95% (F3) and 0.003% (F4). The cumulative percentage for PC1 and PC2 coordinates was over 86.00% so only these 2 PCs had to be considered.

The oils from Columna and Riesling (with a higher content in oleic acid) as well as Merlot and Cabernet Sauvignon (with higher content in linoleic acid) showed a good discrimination on the F1 (PC1) direction; whereas Feteasca Neagra and Cabernet Sauvignon (with high content in SFA) exhibited good discrimination on the F2 (PC2) direction.

A comparison with oils from other seeds seemed of interest. According to the literature the average values for PUFAs (%) (Chira *et al.*, 2011) for oils obtained from sunflower (59.77 ± 4.80), soybean (55.02 ± 2.88) and rapeseed (25.95 ± 1.70) were by far lower than those for the grape seed oil (75.00 ±

2.40%). Thus, this oil stands out for its high content in healthy PUFAs.

3.2.2. Total antioxidant capacity (TAC) of the grape seed oils

Besides FAP, the antioxidant capacity of vegetable oils is essential for their applications (Chambre *et al.*, 2019). The method used to establish the TAC was the CUPRAC assay. This assay is recommended due to its simplicity, reduced cost and reduced reaction time (Apak *et al.*, 2008). It gives an accurate estimation of the TAC of the analyzed sample. The experimental results obtained for the studied oils are presented in Table 1. The oils obtained after the enzymatic treatments of seeds are richer in antioxidants, with the TAC values being 1.7-2.4 times higher compared to those of oils from untreated seeds (see Table 1). The enzymatic treatment destroyed not only the interactions of oil with the cell walls but also that of the antioxidants (Chamorro *et al.*, 2012), increasing the quantities of extracted antioxidants. As expected, the oils obtained from the red grape varieties had higher antioxidant contents and improved health benefits.

A comparison of the TAC of grape seed oils (see Table 1) with other vegetable oil TACs is of interest. Thus, from the literature data the TAC values, expressed as TE (mmol kg^{-1}) are as follows: 1.79 for extra virgin olive oil, 2.20 (soybean), 1.29 (corn), 1.17 (sunflower) (Pellegrini *et al.*, 2003). Due to the enzymatic treatment the related grape seed oils are ranked in better positions by their TE values.

3.3. Complementary possible valorization of other wastes

The processing of grape seeds for oil extraction generates new wastes. Solutions have to be found for further valorization.

An investigation by ^{1}H-NMR (see Figure 4b) of the residue resulting from the concentration of the solutions obtained after enzymatic treatment using a rotavap showed the presence of valuable products.

Occurrence of pectic-oligosaccharides was suggested by the gel aspect of the residue, as well as the specific ^{1}H-NMR peaks at 4.2-4.5 ppm and around the 5.1 ppm (Winning *et al.*, 2007). Other specific peaks are covered by the concentrated commercial enzyme signal (see Figure 4a) and the signal of the citric acid (2.5-2.8 ppm) from the buffer. Small peaks at 6.6-7.5 ppm evidenced the presence of aromatic compounds which were most likely traces of polyphenols (PPs) (Franz *et al.*, 2014). There was no peak at 5.29 ppm in the residue spectrum (Figure 4b), signal characteristic for –CH=CH- of fatty acid (Chambre *et al.*, 2019) as one may see in Figure 5, proving that no oil was lost during pre-treatment.

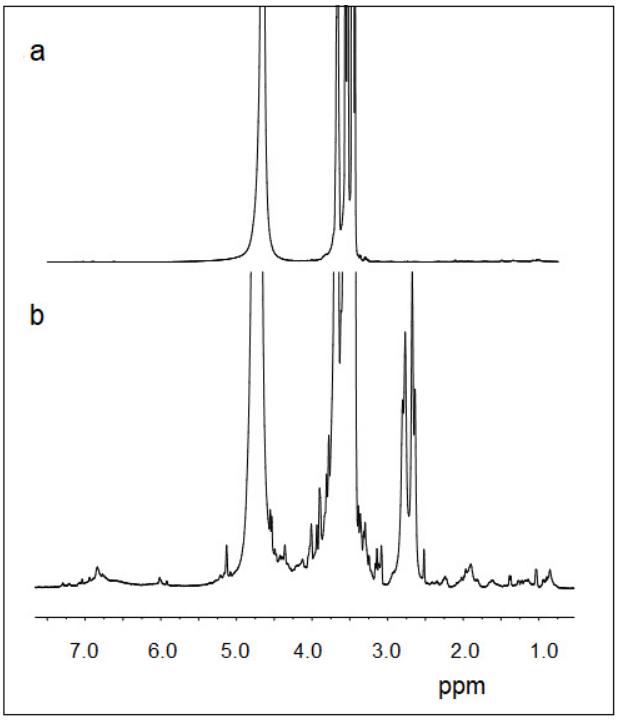

FIGURE 4. ^{1}H-NMR spectra (400 MHz, D_2O, internal standard TSP) for (a) concentrated commercial enzyme; (b) residue obtained by concentrating the solution from the enzymatic pre-treatment

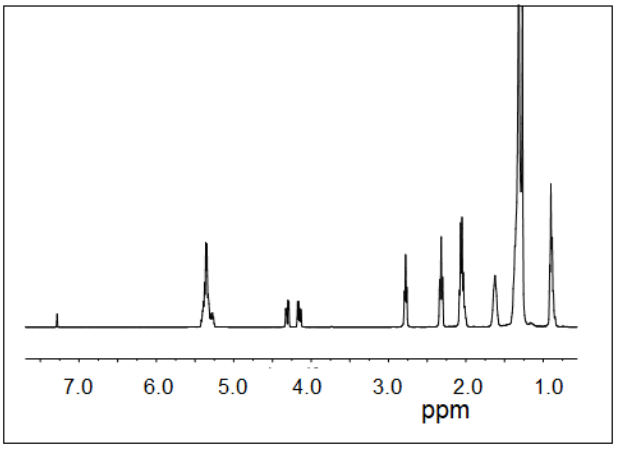

FIGURE 5. ^{1}H-NMR spectrum (400 MHz, D_2O, internal standard TSP) of the oil extracted from the Cabernet Sauvignon seeds

The residual grape seeds from the extraction of oil may be another source for PPs (Maier *et al.*, 2009) as proven by the performed Folin-Ciocalteu assay presented in Table 2.

After the extraction of the oil, the seeds hold over 86% of the initial quantity of PPs, most likely due to the reduced solubility of these compounds in petroleum ether. The literature data claim that vitamin E (tocopherols and tocotrienols) is the main antioxidant in grape seed oils (Wen *et al.*, 2016). This fact was also confirmed by the thermal behavior of grape oils (Chambre *et al.*, 2019).

Further investigation into the valorization of residual grape seed after extraction were also performed by isolating the PPs and using the ligno-cellulosic material for dye adsorption (Tociu, 2019a; Tociu *et al.*, 2019b).

The economic impact of the enzymatic pre-treatment has to be analyzed. Therefore, an accurate EVA analysis should be performed, considering all the by-products from the grape seeds (oil, PPs, adsorbents, char) not only the oil. Thus, new investigations must be carried out.

4. CONCLUSIONS

The paper presents the effect of a *pectin lyase* pre-treatment on grape seeds from six Romanian grape cultivars, raw materials for oil extraction. The following assertions resulted from the experimental work:

The effect of treatment on oil quantity is comparable to processes using cocktails of enzymes.

The statistical analysis indicates the influence of the seed origin on the FAP.

The enzymatic treatment does not affect the FAP of the extracted oils, which contain a high percentage of healthy unsaturated fatty acids.

The enzymatic pre-treatment increased significantly the antioxidant capacity of the extracted grape seed oils.

The antioxidant capacity of the oils is based mostly on vitamin E components as experimentally proven. Most of PPs remain in seeds.

The pre-treatment is performed in a batch system and in mild conditions not needing a complex production unit.

Further investigation into the residual solutions resulting from the enzymatic treatment to recover valuable products, e.g. oligopectins and PPs, are required.

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