

CHARACTERIZATION OF PHYTOCHEMICALS PRESENT IN WINERY BY-PRODUCTS WITH NUTRITIONAL POTENTIAL FOR ITS USE AFTER THE AGRO-INDUSTRIAL PROCESS

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Abstract

The winery by-products contain phytochemicals that are characterized as bioactive components that exhibit nutritional properties. The natural bioactive compounds and nutritional properties of winery's by-products vary with the grape variety and wine making method. The aim of this work was to characterize the nutritional value of grape marc (GM) and yeast biomass (YB) from white wine making using Feteasca Regala variety. Polyunsaturated fatty acids around 67.65% of lipids in GM are essential fatty acids representing phytochemicals with antioxidant properties (omega 6 representing 66.95% of lipids) compared to YB (29.88% and omega 6 representing 24.33% of lipids). YB have a high protein percentage (16.58%) compared to grape marc (7.85%) with high concentrations of essential amino acids, being glutamic acid, aspartic acid, lysine and leucine the most abundant. Infrared FTIR-ATR spectroscopy has been used to determine the functional groups in GM and YB, which show, in addition to the presence of polyphenols, the characteristic compounds that are part of soluble dietary fiber and α and β -glycosidic picks of polysaccharide contents. Due to its phytochemical content, the residues from the wine industry provide essential nutrients for metabolism and can be considered valuable raw materials for their use in animal consumption products.

Key words: grape mark, yeast biomass, phytochemicals, winery's by-products.

INTRODUCTION

The circular economy needs a lot of effort and involvement among companies to create a reliable and usable recovery process for waste and by-products. The management of waste or by-products can create environmental problems that demand revaluation methodologies in order to become sources of wealth thanks to the revalued product (Fernández et al., 2008). Wine cellar by-products such as skins, seeds, and yeast biomass (YB) are important source of phytochemical compounds with high functional value such as proteins, essential fatty acids, fibre, and minerals. In this sense, these by-products could be used as valuable raw materials for human or animal nutrition (Marinescu et al., 2019; Milner, 2004). Feteasca Regala variety is one of the most

cultivated grape varieties in Romania. With a relatively high production rate, it is a very common and highly appreciated variety with a massive production of wine which results in large amounts of by-products. In general, the yield of grape marc (GM) production (by-product obtained after vinification) is about 25-30 kg per 100 kg of grape. From this residue, half are grape skins, 25% stems, and 25% seed (Maicas & Mateo, 2020).

Oenological industry mainly generates solid waste (GM) and liquids (YB) that have a high organic pollutant load and a high concentration of solids. In this case, the legislation is very demanding since it requires a special pre-treatment of this waste. In the case of GM, this is often used to obtain alcohol, and the rest left after this process is usually used as a nutritional supplement in livestock feed. The seeds can be

used to obtain oil and extracts, and the stem can be used in the diet of animals or as biomass. In spite of these advantages, the proteins in grape seeds are considered indigestible, and this is attributed to the strong interaction between proteins and tannins, which limits the digestibility of proteins since tannins are thought to be inhibitors of digestive enzymes (Yu & Ahmedna, 2013). It is known that compared to cereals, the dietary fiber of fruits and vegetables has a higher nutritional quality (Yi et al., 2009). The GM has a significant percentage of dietary fiber, which includes hemicellulose, cellulose, pectin and lignin. Dietary fiber depends on the variety of grapes, white GM having lower fiber concentration (17-28%) than red GM (50-56%), and most of it (95%) is insoluble fiber. Taking into account the large amount of GM that is generated worldwide each year, these have great potential as an important source of insoluble fiber for the development of functional foods (Saura-Calixto, 2011; Yu & Ahmedna, 2013). Grape seeds also contain 7% complex phenols, 16% oil, 11% protein, sugars and minerals. 63% of the total phenols of the red varieties vines are found in the seeds, 34% in the skins and 3% in the must (Murga et al., 2000).

Yeast biomass residue (YB) has a high percentage of alcohol (7%), dietary fiber (20% hemicellulose, 8% cellulose and 13.5% lignin), tartaric acid (20-30%) and proteins (15%). Gomez et al., (2004) shows that YB has a 5.4% lipid content, where palmitic (33%) and linoleic (21%) acids seem to be the most abundant.

FTIR spectroscopy in conjunction with chemometrics represents a valuable tool for monitoring the composition of wine by-products. Grape seeds of grape marc were also studied by FTIR spectroscopy and bioactive compounds were identified as gallic acid carboxylate and proanthocyanidin gallate groups, fatty acid methyl esters, extractable polyphenols in red grape skins as well as other attributes in the grape skin and grape seed (Nogales-Bueno et al., 2017). The ATR-FTIR techniques have been widely used for the analysis of microorganisms to determine their cellular components, such as the cell membrane and wall, thus allowing their classification (Oust et al., 2004). It has also been used to

study the autolysis of *Saccharomyces cerevisiae* in sparkling wines and the biochemical changes in different stages of the alcoholic fermentation process (Burattini et al., 2008; Cavagna et al., 2010).

The winery by-products (GM and YB) can be used in animal feed, for improvement of the capacity against the oxidation of lipids and proteins, as well as for coloring the meat, and also in feeding of the laying hens, improving the size of the egg. In addition, the dietary fiber, which is a group formed by free polysaccharides resistant to enzymatic hydrolysis at the gastrointestinal level, are introduced in the animal diet (DeVries, 2004).

The objective of this study was to determine the content of phytochemicals in the residual biomass of yeast (YB) and grape marc (GM) resulted from the vinification process of the Feteasca Regala variety obtained from the Controlled Designation of Origin of Wines in Pietroasa vineyard, Romania in order to evaluate the nutritional value of these two by-products in animal feed.

MATERIALS AND METHODS

The samples of residual biomass of yeast (YB) and grape marc (GM) resulting from the winemaking process of the white grape variety Feteasca regala (FR) were obtained in 2018 from Controlled Designation of Origin of Wines in Pietroasa vineyard, Romania.

Determination of phytochemical content in YB and GM winery by-products of FR grapes variety

The YB and GM samples taken in the study were distributed in sterile, single-use Petri dishes and placed in the dehydrator (Gorenje FDK24DW food dehydrator) at a temperature of 55°C for 24 h (MARIN et al., 2019). The dried YB and GM samples were analyzed for the content of moisture, crude ash, crude protein, crude fat, total sugars, and total amino acids, according to the requirements specified by the Commission Regulation (EC) 152/2009.

Determination of moisture and ash

The moisture content was determined through drying of the samples at 103°C for four hours, according to Commission Regulation (EC) 152/2009.

The determination of the crude ash (%) was carried out by the gravimetric method, according to Regulation (EC) 152/2009 and the standard SR EN ISO 2171: 2010. It was performed through the ashes of the samples at 550°C.

Determination of crude protein content

The determination of the crude protein content was performed according to the Kjeldahl method, in accordance with the standard SR EN ISO 5983-2: 20101 and the Regulation (EC) 152/2009. The crude protein is obtained based on the nitrogen content determined by the Kjeldahl method multiplied by the factor 6.25.

Determination of total sugar content

The total sugars, expressed as glucose, were determined using the Luff-Schoorl method, in accordance with Commission Regulation (EC) 152/2009.

Determination of crude fat content

The crude fat content was determined based on the Procedure B described in the Regulation (EC) 152/2009, using the extraction with prior hydrolysis.

Determination of NDF and ADF content

The fibrous fractions (g/100g)- the neutral detergent fibre content (NDF) and the acid detergent fibre content (ADF) - were determined by gravimetric determination, using the AOAC Official Method 2002-04 and the AOAC Official Method 973.18, respectively.

Determination of fatty acids and amino acid content

The fatty acids spectrum was determined by GC, using a 456-SCION gas chromatograph configured with FID. The principle of the method consists in the transformation of glycerides from fats and oils into methyl esters by dissolution in organic solvent and transesterification with methanolic potassium hydroxide, followed by the separation of methyl esters from the sample by gas chromatography on capillary chromatographic column with very polar stationary phase. The method is applied to determine the content of fatty acids, including the saturated, monounsaturated and polyunsaturated fatty acids, as well as the omega 3, omega 6, omega 9 fatty acids and *Trans* fatty acids. In addition, the amount of saturated, monounsaturated, polyunsaturated, *Trans* fat, as well as omega 3,

omega 6 and omega 9 fat can be also calculated by reporting the fatty acid content to the extracted fat.

The total amino acids content was determined in accordance with Regulation (EC) No. 152 (2009), after hydrolysis. The working method complies with the standards SR CEN ISO/TS 17764-1:2008 - Animal feeding stuffs - Determination of the content of fatty acids - Part 1: Preparation of methyl esters, and SR CEN ISO/TS 17764-2:2008 - Animal feeding stuffs - Determination of the content of fatty acids - Part 2: Gas chromatographic method.

The content of fatty acids, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, and *Trans* fatty acids is calculated as the sum of the relative mass fractions of the corresponding methyl esters according to the ratio obtained after gas-chromatographic analysis. The result is reported with an accuracy of 0.01% mass fraction.

The amount of saturated fats, monounsaturated fats, polyunsaturated fats, *Trans* fats, omega 3 fats, omega 6 fats and omega 9 fats are determined by reporting the fatty acid content to the fat content of the sample and is expressed in g/100 g product with an accuracy of 0.01 g/100 g.

Spectroscopic analysis for YB and GM winemaking residue powdered samples from FR grapes variety

Infrared spectroscopy (ATR-FTIR) was used to qualitatively determine the chemical functional groups of two winemaking by-products powdered samples, yeast biomass residue (YB) and grape marc residue (GM) from white Feteasca Regala (FR) grapes variety. No other sample preparation was performed for spectral analysis. The spectra of each sample were collected with a Thermo Scientific (Waltham, MA, USA) Nicolet iS50 FT-IR spectrometer equipped with an inbuilt diamond attenuated total reflection (ATR) system with a refractive infrared beam bouncing off a 45 angle of incidence. The instrument was fitted with OMNIC software (OMNIC 7.3, Thermo Fisher Scientific Inc.). Before measuring each sample of vinification residues, the background was carried out. The spectral range for each sample was between 400-4000 cm^{-1} with an average of 64 scans and a resolution of 8 cm^{-1} at a scanner velocity of 7.5 kHz.

Statistical analysis

Each sample analysis was worked out in triplicate. The data reported are means (\pm SD) of at least three independent experiments.

RESULTS AND DISCUSSIONS

Although the phytochemicals content is higher in grapes, in GM and YB by-products generated in winemaking they are also present, since part of them are transmitted to the wine during the vinification process (Alonso et al., 2002; Spanghero et al., 2009). The content depends on the grape variety and is influenced by location, climate, and state of maturity of the grapes and the fermentation process, as well as by the processing and production of wine (red or white grapes, extraction, drying etc.) (Ojeda et al., 2002; Muñoz et al., 2004; Baumgärtel et al., 2007).

Phytochemical content in YB and GM winery by-products of white grapes FR variety

Table 1 presents the phytochemical compositions of YB and GM of the white grape FR variety. Both powder residues GM and YB have a dry matter content of approx. 95%, and the ash content was higher in YB residue (14.92%) than in GM residue (4.15%).

Table 1. Biochemical compositions of winery by-products (YB and GM) from FR grapes variety

Parameters	YB	GM
Ash (%)	14.92	4.15
Moisture (%)	3.87	7.49
CP (%)	16.58	7.85
TS (g/100 g)	0.71	20.00
Lipids (g/100 g)	3.30	5.23
NDF (g/100 g)	16.15	41.01
ADF (g/100 g)	4.80	32.55

Values are expressed as mean; FR: Feteasca Regala variety; YB: yeast biomass; GM: grape marc; CP: crude protein; TS: total sugar; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber.

The CP content in YB was twice higher (16.58%) than in the GM residue (7.85%), but all the other parameters analysed presented higher values in the GM residue than in the YB residue, highlighting TS content (20 g/100 g) and dietary fiber values (71.74% NDF, respectively 87.14% ADF for the total). The winery by-products can be used as feed, knowing that there are differences between the

nature of dietary fiber, cereals being the main sources of cellulose and hemicellulose, fruits and vegetables are mainly pectin (Elleuch et al., 2011). The cell wall specific carbohydrates (polysaccharide) (e.g. β -glucans) have not been determined and therefore these are not included in the composition presented in table 1.

Other authors highlight that GM contains 40% fermentable carbohydrates (by dry weight) from white grapes and 4.6% from red grapes (Llobera et al., 2007). Dietary fiber has different components with physical, chemical and physiological properties, such as oligosaccharides, lignin, resistant starch and associated plant substances and depends on the grape variety (17.3-28% white grape and 51.1-56.3% red grape) (Deng et al., 2011; Bravo and Saura-Calixto, 1998).

Fatty acids and their profile in YB and GM winery by-products of white grapes FR variety

Both content and composition of fatty acids in GM and YB depend on the variety and maturity of the grape (Yu and Ahmedna, 2013; Bravi et al., 2007). GM highlighted for its PUFA content (67.65% lipids), omega 6 acids represented 98.96% of PUFAs, compared to YB (PUFA 29.88% of lipids of which 81.42% were omega 6 acids). In the case of omega 3 acids, the values were higher (5.55% of lipids) in YB residue and in GM residue were almost non-existent or at very low values (0.70% of lipids) (Table 2).

Table 2. Total fatty acid contents (% of lipids) in winery by-products (YB and GM) from FR grapes variety

Fatty acids (% of lipids)	YB	GM
Σ SFA	30.14	12.88
Σ MUFA	23.26	19.38
Σ PUFA	29.88	67.65
Σ FAT	4.18	0.82
Σ acid C18:2 trans	3.23	nd
Σ acid C18:3 trans	nd	0.09
Σ Ω 3	5.55	0.70
Σ Ω 6	24.33	66.95
Σ Ω 9	14.95	18.17

Values are expressed as mean; FR: Feteasca Regala variety; YB: yeast biomass; GM: grape marc; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; FAT: Fatty acid Trans; nd: Not detected.

The profile of the content of fatty acids in the residues of GM and YB of white grapes of the FR variety is presented in Table 3.

Table 3. Profile of the fatty acid contents (% of lipids) in winery by-products (YB and GM) from FR grapes variety

Fatty acids (% of lipids)	YB	GM
Caprinic acid (C10:0)	0.58	nd
Lauric acid (C12:0)	0.94	nd
Myristic acid (C14:0)	0.87	0.18
Myristoleic acid (C14:1)	0.25	nd
Pentadecanoic acid (C15:0)	0.06	nd
Palmitic acid (C16:0)	16.61	8.32
Palmitoleic acid (C16:1)	6.85	0.39
Margaric acid (C17:0)	0.13	nd
Cis Heptadecenoic acid (C17:1)	0.14	nd
Stearic acid (C18:0)	6.74	4.00
Oleic acid (C18:1 cis)	14.78	17.69
Vaccenic acid (C18:1 trans)	0.91	0.82
Linoleic acid (C18:2 cis)	19.84	66.95
α -Linolenic acid (C18:3n-3)	4.59	0.70
γ -Linolenic acid (C18:3n-6)	4.33	nd
Arachidic acid (C20:0)	1.59	0.26
Eicosenoic acid (C20:1 n-9)	0.13	0.15
Eicosadienoic acid (C20:2 n-6)	0.06	nd
Eicosatrienoic acid (C20:3 n-3)	0.14	nd
Arachidonic acid (C20:4n-6)	0.10	nd
Eicosapentaenoic acid (C20:5n-3)	0.58	nd
Heneicosanoic acid (C21:0)	0.33	nd
Docosanoic (Behenic)acid (C22:0)	1.48	0.12
Docosapentaenoic acid (C22:5 n3)	0.24	nd
Tricosanoic acid (C23:0)	0.24	nd
Lignoceric acid (C24:0)	0.53	nd
Nervonic acid (C24:1)	Nd	0.33
Caprylic acid C8:0 -	0.04	nd

Values are expressed as mean; FR: Feteasca Regala variety; YB: yeast biomass; GM: grape marc; nd: Not detected.

The results show that in GM, due to the high content of linoleic acid (66.95% of lipids), there is a high content of omega 6 fatty acids, due to the presence of the seeds (Marin et al., 2019). The main essential fatty acids found are linoleic (66.95% of lipids), oleic (17.69% of lipids), palmitic (8.32% of lipids) and stearic (4.00% of lipids). The YB residue contains fatty acids in a lower quantity but a wider variety and the α -linolenic acid and γ -linolenic acid contents are higher (4.59%, respectively 4.33% of lipids) than in GM (0.70% of lipids, respectively not detected), belonging to acids omega 3. It is remarkable that unsaturated fatty acids predominate, representing an important source of essential fatty acids for the feed industry (Marin et al., 2019; Barbulescu et al., 2020; Teodorescu et al., 2020).

The incorporation of grape marc in the diet of ruminants, as shown by Jerónimo et al. (2012), can interfere with the lipid profile of meat due to the presence of active phytochemical

components. The author observed an increase in unsaturated fatty acids (long chain n6) when including 25 g kg⁻¹ dry matter of grape seed extract in lamb diets. Another study demonstrates a lower level of oxidation in turkey meat hamburger by ingestion or post-mortem addition of grape seed extract, and also blue fish supplemented with grape seed extract (100 ppm) keeps its freshness for 10 days of conservation at 4°C, in comparison with the control (3 days) (Cagdas & Kumcuoglu, 2015).

Amino acids content in YB and GM winery by-products of white grapes FR variety

Rao (1994) and Zhou et al. (2010) showed that the concentration and composition of amino acids vary with the variety of the grape, growing area, fertilization etc. Our previous studies showed slightly higher values of total essential amino acids in GM residues of the red varieties compared to white varieties (1 g/100 g difference) (Marin et al., 2019). In the case of the FR variety and for the two residues analyzed, higher values were observed in YB residues compared to GM residues (table 4). Essential amino acids, important in nutrition (Castro Sousa et al., 2014), the most abundant found were aspartic acid (1.54 g/100 g, respectively 8.87 g/100 g), glutamic acid (1.35 g/100 g, respectively 1.08 g/100 g), serine, alanine and leucine.

Table 4. Essential amino acids content (g/100g) in winery by-products (YB and GM) from FR grapes variety

Essential amino acids (g/100 g)	YB	GM
Aspartic acid	1.54 ±0.19	0.87 ±0.11
Threonine	0.85 ±0.10	0.43 ±0.05
Serine	1.33 ±0.16	0.73 ±0.09
Glutamic acid	1.35 ±0.17	1.08 ±0.13
Proline	0.60 ±0.07	0.76 ±0.09
Glicine	0.83 ±0.10	0.65 ±0.08
Alanine	0.94 ±0.12	0.46 ±0.06
Valine	0.70 ±0.09	0.24 ±0.03
Isoleucine	0.66 ±0.08	0.46 ±0.06
Leucine	1.10 ±0.13	0.92 ±0.11
Tyrosine	0.60 ±0.07	0.11 ±0.02
Phenylalanine	0.60 ±0.07	0.30 ±0.04
Lysine	1.10 ±0.13	0.41 ±0.05
Histidine	0.35 ±0.04	0.19 ±0.02
Arginine	0.62 ±0.07	0.46 ±0.06

Data are expressed as mean ± SD; FR: Feteasca Regala variety; YB: yeast biomass; GM: grape marc.

Spectroscopic analysis of YB and GM winery by-products of white grapes FR variety

FTIR spectroscopy is a suitable tool for applications in the oenological sector (Lucarini et al., 2020). Fourier transform infrared spectroscopy (ATR-FTIR) was used for qualitative analysis of the YB and GM residue samples obtained in the vinification process of the white grape FR variety (Figures 1 and 2).

The differences between the samples of the two residues (YB and GM) were determined with the help of the peaks found in the spectral region of 4000-500 cm^{-1} (Basalekou et al., 2015). Table 5 collect the detailed peak positions and band assignments to compare the two residues.

Our study compared the specific adsorption profiles identified between the two residues analyzed in which the group's functional characteristics absorb for each of the residues. In general, the spectres seem to have the peaks in the same areas with respect to the positions of the spectral bands, thus we can identify the

signatures of the main functional groups for the two residues. Differences can be seen between the shapes of the bands and the relative intensities in the spectra.

In the case of YB residue, irradiation with infrared light at different wavelengths exhibited well-defined spectral regions corresponding to the vibration determined by the components of the cells (proteins, fatty acids, polysaccharides, chitins, glucans among others) Grangeteau et al. (2015 and 2016). Characteristic vibrations were observed with very sharp peaks due to proteins in the spectral region 1621 cm^{-1} (amide I) and 1540 cm^{-1} (amide II), 1131 cm^{-1} (nucleic acids) and for carbohydrates the bands between 903-1299 cm^{-1} representing the content of α and β -glucans. β -glucans of a typical yeast cell consist of $\sim 30\text{-}45\%$ of β -1, 3 glucan and $\sim 5\text{-}10\%$ of β -1, 6 glucan (Soares and Soares, 2012). β -glucans, the main components of yeast biomass, enhance the innate immune response, since mammalian cells lack this component (Chan et al., 2009).

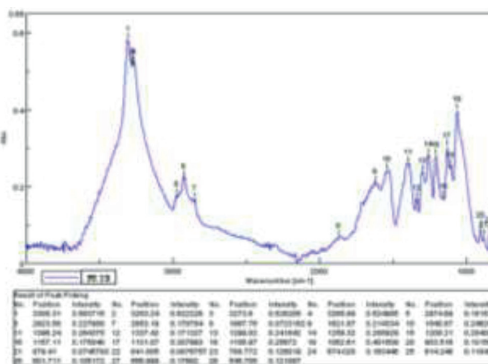


Figure 1. ATR-FTIR spectral analysis of the winemaking by-products (YB) from FR grapes variety

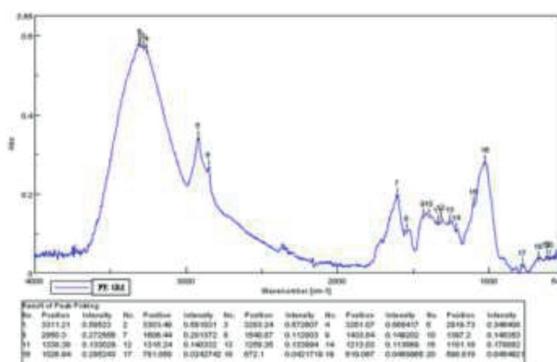


Figure 2. ATR-FTIR spectral analysis of the winemaking by-products (GM) from FR grapes variety

Table 5. Wavenumber (cm⁻¹) assignments of ATR-FTIR spectra of YB and GM powders from FR grapes variety with different sizes

Wavenumber (cm ⁻¹)				Assignments
YB		GM		
Positions	Intensity	Positions	Intensity	
3308	0.5837	3311	0.5852	
3283	0.5222	3303	0.5810	-OH stretch vibration, -H bonded vOH=3200-3600 cm ⁻¹
3273	0.5262	3283	0.5226	
3265	0.5249	3261	0.5654	
2976	0.1815			
2926	0.2279	2919	0.3464	C-H stretching, aliphatic
2853	0.1707			
		2350	0.2725	
1621	0.2143			Aromatic CH bonds (COO ⁻)
		1606	0.2013	
1540	0.2462	1540	0.1120	C = C stretching bands
		1433	0.1492	Antisymmetric in-plane bending of -CH ₃
1396	0.2643	1397	0.1483	Symmetric in-plane bending of -CH ₃
1337	0.1713	1315	0.1403	
1299	0.2418			
1259	0.2859	1258	0.1338	Stretching vibration of C-O
1208	0.2840	1213	0.1136	
1131	0.3079			
1106	0.2567	1101	0.1788	
1062	0.4015			Stretching vibration of C-O
		1026	0.2852	
903	0.1015			
788	0.1260	781	0.0242	CH out of-plane conformations
674	0.1184	672	0.0421	
601	0.1051			
566	0.1769			
545	0.1210			

Also peaks with high absorption intensity were found in the spectral region 3308-3265 cm⁻¹ (NH of proteins), 2976-2853 cm⁻¹ (CH of lipids and proteins).

GM residue analyzed by ATR-FTIR showed the presence of absorption peaks at 3311-3261, 2919, 2350, 1606, 1540, 1433, 1397, 1258, 1101, 1026, 781 and 672 cm⁻¹. The bands with the most absorbing peaks are found at 3311, 3303, 3283 and 3261 cm⁻¹ corresponding to the -OH stretching vibration of the phenolic structures. At the 2919 cm⁻¹ absorption peak, this is due to symmetric and asymmetric C-H stretching bonds in the CH₂ and CH₃ groups, methyl band bonds belonging to lipids (Ricci et al., 2015). The 1433-781 cm⁻¹ region represents

the *fingerprint* that provides important information on the organic compounds (sugars, alcohols, and organic acids) present in the sample. The food industry and farming should interact each other to benefit both from this interaction, since both have problems disposing of their waste. By-products of the winery, it is possible to use them as feed with antioxidant functions, improving their behaviour against the oxidation of lipids and proteins, and the colour of the meat (Jerónimo et al., 2012).

CONCLUSIONS

Our study shows a high content of polyunsaturated fatty acids (PUFA) in the main

residues of the winery, GM (67.65% of lipids) and YB (29.88% of lipids). Among these the most important and with the highest content is linoleic and oleic acid (66.95%, respectively 17.69% of lipids) in GM, which are precursors for omega 6 fatty acids, due to the presence of the seeds. Precursors for fatty acids omega 3 were observed in YB as well, α -linolenic acid and γ -linolenic acid (4.59%, respectively 4.33% lipids). The results showed a higher dietary fiber GM content (41.01 g/100 g NDF and 32.55 g/100 g ADF) compared to YB content (16.15 g/100 g NDF and 4.80 g/100 g ADF). Due to the content of dietary fiber in grape marc, with a high content of extractable and non-extractable phenolics, this is important in lipid metabolism.

ATR-FTIR provides molecular vibrations (stretching, bending and torsion) of the phytochemicals present in the by-products of the winery (GM and YB) representing the molecular *fingerprint* of the sample. The YB residue spectra are well defined with very marked peaks, representing cell components: 1621 cm^{-1} (amide I); 1540 cm^{-1} (amide II); 1131 cm^{-1} (nucleic acids) and 903-1299 cm^{-1} (carbohydrates; α and β -glucans). Peaks very sharp bands between 3308-3265 cm^{-1} represent stretching NH of proteins and CH of lipids and proteins. The peaks of the GM residue recognize the -OH stretching of phenolic structures, polysaccharides and/or lignin (3311-3261 cm^{-1}) This residue contains grape seeds with beneficial PUFA fatty acid content, demonstrated by stretching vibrations (2919 cm^{-1}) symmetric and asymmetric CH (CH_2 and CH_3). Study of the content in phytochemicals of YB and GM demonstrated the antioxidant properties of these residues, in addition to providing essential nutrients, in addition to providing essential nutrients for the proper functioning of the metabolism with a healthy effect in the medium and long term.

ACKNOWLEDGEMENTS

This work was supported by the project 20PFE/2018 "Development of the Center for the superior valorisation of the by-products from the vineyard farms", Program 1 -Development of the National Development Research System, Subprogram 1.2 -Institutional Performance - Institutional Development

Projects - Funding Projects for Excellence in Institutional Development Research, PNCDI III.

REFERENCES

- Alonso, A.M., Guillén, D.A., Barroso, C.G., Puertas, B., & García, A. (2002). Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. *Journal of Agricultural and Food Chemistry*, 50, 5832–5836.
- Basalekou, M., Pappas, C., Kotsieridis, Y., Strataridaki, A., Geniatakis, E., Tarantilis, P.A., & Kallithraka, S. (2015) Monitoring wine aging with Fourier transform infrared spectroscopy (FT-IR). *BIO Web of Conferences*, 5.
- Barbulescu, I.D., Teodorescu, R.I., Dragotoiu, D., Cimpeanu, S.M., et al. (2020). Feed ingredient from dried grape marc. Demand patent A/00303/02.06.2020
- Baumgärtel, T., Kluth, H., Epperlein, K., & Rodehutsord, M. (2007). A note on digestibility and energy value for sheep of different grape pomace. *Small Ruminant Research*, 67, 302–306.
- Bravi, M., Spinoglio, F., Verdone, N., et al. (2007). Improving the extraction of α -tocopherol-enriched oil from grape seeds by supercritical CO_2 . Optimisation of the extraction conditions. *Journal of Food Engineering*, 78, 488–493.
- Bravo, L., & Saura-Calixto, F. (1998) Characterization of dietary fiber and the in vitro indigestible fraction of grape pomace. *American Journal of Enology and Viticulture*, 49, 135–141.
- Burattini, E., Cavagna, M., Dell'Anna, R., Malvezzi Campeggi, F., Monti, F., Rossi, F., & Torriani, S. (2008). A FTIR microspectroscopy study of autolysis in cells of the wine yeast *Saccharomyces cerevisiae*. *Vibrational Spectroscopy*, 47, 139–147.
- Castro Sousa, E., Uchôa Thomaz, A.M.A., Beserra Carioca, J.O., De Moraes, S.M., et al. (2014). Chemical composition and bioactive compounds of grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semi-arid region of Northeast Brazil, 135. *Food Sci. Technol. Campinas*, 34(1), 135–142.
- Cavagna, M., Dell'Anna, R., Monti, F., Rossi, F., & Torriani, S. (2010). Use of ATR-FTIR microspectroscopy to monitor autolysis of *Saccharomyces cerevisiae* cells in a base wine. *Journal of Agricultural and Food Chemistry*, 58, 39–45.
- Chan, G. C., Chan, W. K., & Sze, D. M. (2009). The effects of beta-glucan on human immune and cancer cells. *Journal of Hematology & Oncology*, 2(25), 1–11.
- Deng, Q., Penner, M.H., & Zhao, Y.Y. (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Research International*, 44, 2712–2720.
- DeVries, J.W. (2004) Dietary fiber: The influence of definition on analysis and regulation. *Journal of AOAC International*, 87, 682–706.

- Cagdas, E., & Kumcuoglu, S (2015) Effect of grape seed powder on oxidative stability of precooked chicken nuggets during frozen storage. *J. Food Sci. Technol.*, 52, 2918–2925.
- Elvira-Torales, L. I., Martín-Pozuelo, G., González-Barrio, R., Navarro-González, I., Pallarés, F. J., Santaella, M., García-Alonso, J., Sevilla, Á., & Periago-Castón, M. J. (2019). Ameliorative Effect of Spinach on Non-Alcoholic Fatty Liver Disease Induced in Rats by a High-Fat Diet. *International journal of molecular sciences*, 20(7), 1662.
- Fernández, M.P., Rodríguez, J.F., García, M.T., de Lucas, A., & Gracia, I. (2008) Application of supercritical fluid extraction to brewer's spent grain management. *Industrial & Engineering Chemistry Research*, 47, 1614–1619.
- Jerónimo, E., Alfaia, C.M.M., Alves, S.P., Dentinho, M.T.P., Prates, J.A.M., Vasta, V., Santos-Silva, J., & Bessa, R.J.B. (2012). Effect of dietary grape seed extract and *Cistus ladanifer* L. in combination with vegetable oil supplementation on lamb meat quality. *Meat Science*, 92, 841–847.
- Llobera, A., & Cañellas, J. (2007). Dietary fiber content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): Pomace and stem. *Food Chemistry*, 101, 659–666.
- Lucarini, M., Durazzo, A., Kiefer, J., Santini, A., Lombardi-Boccia, G., Souto, E.B., Romani, A., Lampe, A., Ferrari Nicoli, S., Gabrielli, P., Bevilacqua, N., Campo, M., Morassut, M., & Cecchini, F. (2020). Grape Seeds: Chromatographic Profile of Fatty Acids and Phenolic Compounds and Qualitative Analysis by FTIR-ATR Spectroscopy. *Foods*, 9, 10.
- Maicas, S., & Mateo, J.J. (2020). Sustainability of Wine Production. *Sustainability*, 12, 559.
- Marin, S., Bărbulescu, I.D., Perte, A.M., Matei, P.M., Marinescu, S.I., Teodorescu, R.F., Marin, D., Dumitrache, C., Tudor, V., & Teodorescu, R.I. (2019). Valorisation of GM from Pietroasa vineyard for obtaining new dry feed ingredients as a potential polyphenols source. *Scientific Papers. Series B Horticulture*, LXIII(1).
- Marinescu, S.I., Marin, D.E., Jókai, Z., Üveges, M., Albu Kaya, M., Bunduc, V., Dernovics, M., Hingyi, H., Begea, M., & Bărbulescu, I.D. (2019). Bio-ingredients based on spent industrial yeast biomass. *Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering*, VIII(8), 196–201.
- Milner, J.A. (2004). Molecular targets for bioactive food components. *Journal of Nutrition*, 134(9), 2492S–2498S.
- Muñoz, O., Sepúlveda, M., & Schwartz, M. (2004). Effects of enzymatic treatment on anthocyanic pigments from grapes skin from Chilean wine. *Food Chemistry*, 87, 487–490.
- Murga, R., Ruiz, R., et al. (2000). Extraction of natural complex phenols and tannins from grape seeds by using supercritical mixtures of carbon dioxide and alcohol. *Journal of Agricultural and Food Chemistry*, 48(8), 3408–3412.
- Nogales-Bueno, J., Baca-Bocanegra, B., Rooney, A., Hernández-Hierro, J.M., Heredia, F.J., & Byrne, H.J. (2017). Linking ATR-FTIR and Raman features to phenolic extractability and other attributes in grape skin. *Talanta*, 167, 44–50.
- Nogales-Bueno, J., Baca-Bocanegra, B., Rooney, A., Hernández-Hierro, J.M., Byrne, H.J., & Heredia, F.J. (2017). Study of phenolic extractability in grape seeds by means of ATR-FTIR and Raman spectroscopy. *Food Chem.*, 232, 602–609.
- Ojeda, H., Andary, C., Creaba, E., Carbonneau, A., & Deloire, A. (2002). Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* var. Shiraz. *American Journal of Enology and Viticulture*, 53, 261–267.
- Oust, A., Moretto, T., Kirschner, C., Narvhus, J. A., & Kohler, A. (2004). FT-IR spectroscopy for identification of closely related lactobacilli. *Journal of Microbiological Methods*, 59, 149–162.
- Ricci, A., Olejar, K.J., Parpinello, G.P., Kilmartin, P.A., & Versari, A. (2015). Application of Fourier Transform Infrared (FTIR) Spectroscopy in the Characterization of Tannins. *Appl. Spectrosc. Rev.*, 50, 407–442.
- Saura-Calixto, F. (2011). Dietary fiber as a carrier of dietary antioxidants, an essential physiological function. *Journal of Agricultural and Food Chemistry*, 59, 43–49.
- Soares, E. V., & Soares, H. M. (2012). Bioremediation of industrial effluents containing heavy metals using brewing cells of *Saccharomyces cerevisiae* as a green technology: a review. *Environmental Science and Pollution Research International*, 19(4), 1066–1083.
- Spanghero, M., Salem, A.Z.M., & Robinson, P.H. (2009). Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. *Animal Feed Science and Technology*, 152, 243–255.
- Teodorescu, R.I., Bărbulescu, I.D., Dragotoiu, D., Cimpeanu, S.M. et al. (2020). Biotechnological proteic ingredient based on residual yeasts obtained from the vinification process, demand patent A/00304/02.06.2020
- Van Soest, P.J., Robertson, J.B., & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583–3597.
- Yi, C., Shi, J., Kramer, J., Xue, S., Jiang, Y., Zhang, M., Ma, Y., & Pohorly, J. (2009). Fatty acid composition and phenolic antioxidants of winemaking pomace powder. *Food Chemistry*, 114, 570–576.
- Yu, J., & Ahmedna, M. (2013). Functional components of grape pomace: their composition, biological properties and potential applications. *International Journal of Food Science & Technology*, 48(2), 221–237.