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Original paper

Nutritive importance of by-products from winemaking process for feed industry interest

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Abstract

The objective of this work was to study the nutritional composition of winemaking by-products as grape marc (GM) of the varieties Tamaioasa romaneasca (TMGM), Burgund mare (BMGM), Merlot (MGM) and Riesling Italian (RIGM) cultivated under the Controlled Designation of Origin (DOC) of Wines in Pietroasa vineyard, Romania. GM samples were conditioned in a forced air oven at 55°C for 24 h. Dry matter, crude protein, cellulose, crude ash, crude lipids, fatty acids and amino acids of the GM by-products from the winemaking processes were analysed. TRGM variety showed the highest amount of total amino acids compounds (8.5 g/100 g) compared to RIGM variety (7.7 g/100 g). A source of linolenic acid (omega-3 fatty acids) was found for BMGM variety (0.31 g/100 g total FAME) and linoleic acids (omega-6 fatty acids) for the MGM (63.54 g/100 g total FAME) and RIGM (61.39 g/100 g total FAME) samples. Infrared spectroscopy (ATR-FTIR) was used to qualitatively determinate the chemical functional groups of GM samples. Differences between the samples spectra in region of 1800-700 cm^{-1} (*fingerprint*) were detected. The results prove the importance of these added-value by-products with great applicability in feed industry.

Keywords Grape marc, Pietroasa vineyard, winemaking by-product, nutritive compounds.

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Introduction

Vitis vinifera L. is a plant that is grown worldwide for grapes, consumed fresh or pressed to make beverages. The processing of the grapes generates a large number of by-products that can be classified as follows: solid by-products (stems, seeds, skins and pulp), high-viscosity by-products (leverage) and low viscosity products (wastewater) (BEKHIT et al, 2016 [4]; FAO, 2010 [16]). GM (or grape pomace) is the main fraction of solid by-products, representing up to 60% of its weight and 20% to 25% of the received grape (SPIGNO et al, 2017 [31]).

The grape production is the second largest production in the world, of which only 80% is used in the wine industry and 20% represents the grape marc. GM consists of the skin, seeds and stems with a high content of phenolic compounds, lipids, polysaccharides and proteins. GM being an abundant by-product, its valorisation represents great economic interest (DENG et al, 2011 [9]; SCHIEBER et al, 2001 [30]; FAO, 2016 [12]). According to the estimations, one kilogramme of GM is generated for each six litres of wine (MENDES et al, 2013 [21]). One tonne of GM is composed of around 430 kg of grape skins, 250 kg of grape stalks and 230 kg of grape seeds (NERANTZIS and TATARIDIS, 2006 [24]). The GM amount manufactured per tonne of grapes depends on the type of press used for pressing whole grapes, terroir and the variety of the grape (BACIC, 2003 [2]).

According to FÉVRIER et al (2009) [14] grape marc is a fresh (around 65-68% water) and perishable product, and must be dried before being used for feeding or silage. The drying process is generally carried out in large rotary drum dryers where the hot air, forced through the pump, expelling the gases and moisture vapour from the dryer. EL BOUSHY et al (2000) [10] proposes the addition to the dry and milled pomace a small amounts of molasses to improve its energy content as well as lime to help bind the pectin and raise the pH. As a drying method, in a process described in Australia, the grape marc is kept for 20 minutes in a rotating drum with gas heated to a temperature of 120°C. Resulting GMs dried are ground and then steam-pelleted at 85°C (MOATE et al, 2014 [22]). Organic by-products obtained from the Bordeaux grape (*Vitis labrusca* L.), namely grape skin flour (GSF) and grape pomace flour (GPF), which is mainly composed of partially defatted seeds and grape skin fragments, were ground and sieved (28 mesh) to standardize the particle size. The results obtained by KARNOPP et al (2017) [19], show that the GPF and GSF from the organic grape juice industry are potential materials for use by food companies. ELKAHOUI et al (2018) [11] studied air-dried pomace skin of four grape cultivars Carignan, Merlot, Cabernet Sauvignon, and Syrah. The phenolic compound of GM skin, a winemaking by-product, was tested. The quantity from GM of total dietary fibres (46.17g/100g) noticed quantitatively compared to the concentration of carbohydrate (29.2 g/100 g), proteins (8.49 g/10 g), and lipids (8.16 g/100 g). The total energy was 224 Kcal/100 g.

Infrared (IR) spectroscopy is the most appropriate method of identifying the presence of polar functional groups in the structure of molecules of organic compounds. Spectroscopic techniques have great advantages for studying the physicochemical nature of materials (composition-structure) due to their analytical speed, their non-destructive nature and their versatility of use. The Attenuated Total Reflection (ATR) phenomenon consists of the passage of the IR beam from an optically denser medium with higher refraction index to a thinner medium after the sample is taken in close proximity to the ATR crystal surface (MOVASAGHI et al, 2007 [23]; BASALEKOU et al, 2015 [3]). Four samples of white and red GMs have been studied in order to determine the infrared spectrum and we have been able to identify the peaks with their corresponding vibrations.

In the present work, GM by-products have been studied to attempt their use in the feed industry as a source of protein, cellulose, lipids and amino acids.

Materials and Methods

White and red samples grape marc of Tamaioasa romaneasca (TRGM), Riesling Italian (RIGM), Merlot (MGM) and Burgund mare (BMGM) varieties investigated were obtained in 2017 from Controlled Designation of Origin of Wines in Pietroasa vineyard, Romania.

a. Grape marc dehydration (GM)

The GMs 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 until it reached a humidity of 5% (MARIN et al, 2019 [20]).

b. Chemical analyses for dried GM samples

The determination of the dry matter content of the GMs was carried out by the gravimetric method, after removal of the water by evaporation in an oven at 103°C. The by-products were ground and sieved (mesh 28) to standardize the particle size and weighed on an analytical balance.

The nutritional substances in the fodder can be determined by destructive methods (Weende and Van Soest schemes), or non-destructive (near-infrared spectroscopy). The Weende scheme was developed in 1857. According to this scheme, protein, cellulose, fat and ash were determined.

The nitrogen content determined by the Kjeldahl method is multiplied by the factor 6.25 and the *crude protein content* is obtained (value 6.25 resulting from the admittance of the average nitrogen content, in the feed protein, equal to 16%).

The analysis of the *crude lipids content* was performed by the organic solvent extraction method. Mass determination of the sample was subject to extraction with an organic solvent.

Crude cellulose content was measured by the intermediate filtration method. Mass determination of the

sample was subject to successive boiling with solutions of sulfuric acid and sodium hydroxide. The residue is filtered, dried, calcined and weighed.

The *crude ash content* was analysed through the gravimetric method.

c. Method of determining fatty acids and amino acid content for dried GM samples

The equipment used is the HPLC Surveyor Plus system, Thermo Electron, equipped with a PDA detector. The separation was performed on Hypersil BDS (Base Deactivated Silica) C_{18} chromatographic column, with silica gel, with dimensions 250 4.6 mm, and the particle size of 5 µm.

The method consists of the transformation into *fatty acid* methyl esters of the samples of dehydrated GMs subjected to the analysis, followed by the separation of the components in the capillary chromatographic column. Identification was done by comparison with standard chromatograms and quantitative determination of fatty acids (expressed as g/100g total FAME).

The method for the determination *amino acid content* was in accordance with the Commission Regulation (EC) No 152/2009 of 27 January 2009 establishing sampling and analyzing methods for official feed control, Official Journal of the European Union 26.02.2009 EN L45/1 and AOAC Official Methods of Analysis (2005) [25]. Quantitative determination of amino acids is carried out in two stages. The first step is that of the hydrolysis of the peptide bonds existing between the constituent amino acids of the protein, in the case of determination of amino acids with sulphur; the hydrolysis step is preceded by oxidation. The second step is the determination (dosage) of the amino acids released following the hydrolysis of the peptide bonds. In turn, this step requires several steps: the individual separation of the amino acids from the existing mixture in the protein hydrolysate by high performance liquid chromatography (HPLC), their detection and quantification. The results obtained for the determination of amino acids by high performance liquid chromatography (HPLC) are expressed in g/100 g sample as such.

d. Spectroscopic analysis for dried GM samples

Spectra of four GM powder for white (TRGM and RIGM) and red (MGM and BMGM) samples were collected with a Thermo Scientific (Waltham, MA, USA) Nicolet iS50 FT-IR Spectrometer. The spectrophotometer was equipped with an inbuilt diamond attenuated total reflection (ATR) system with a refractive infrared beam bouncing off a 45 angle of incidence. Background measurement was taken before each sample measurement. The spectrum of each sample was obtained in the 400-4000 cm^{-1} range and the average of 64 scans at a resolution of 8 cm^{-1} with a scanner velocity of 7.5 kHz was recorded. The instrument was fitted with OMNIC software (OMNIC 7.3, Thermo Fisher Scientific Inc.). No further sample preparation was done for spectral analysis.

Results and Discussions

GM was frequently considered as agricultural waste and discarded; in our work we were trying to demonstrate that it represents a feasible source of nutritional substances with great potential to be used as a functional ingredient, as a by-product in the feed industry.

a. Chemical composition of dried GM samples

The TRGM (9.9%), MGM (9.6%) and BMGM (9.5%) present a higher percentage of proteins than that of the RIGM (8.6%). The pomace in red and aromatic winemaking is macerated with the must until after the alcoholic fermentation and in addition to the degradation of sugars, it occurs the transformation of the main part of the nitrogenous matter, necessary for the multiplication of yeasts, favouring the increase of nitrogenous matter during the process. On the other hand, the pomace in white winemaking is made up of grape remains and bunch; this component of the bunch is generally poor in nitrogenous substances, as well as in proteins. Our results are in accordance with the one reported by TSENG and ZHAO (2013) [32]), as shown in Table 1.

Table 1. The results of the determinations in the WEENDE scheme, obtained for the GM samples under analyses

Sample	Dry matter (%)	Crude protein (%)	Crude lipids (%)	Cellulose (%)	Ash (%)
TRGM	92.3	9.9	5.9	23.9	4.8
RIGM	90.3	8.6	4.8	23.5	4.6
MGM	91.6	9.6	8.5	26.0	5.3
BMGM	93.9	9.5	7.3	26.9	5.3

Cellulose content is higher in GM red varieties compared to GM white varieties, knowing that the by-products of the red varieties have higher hemicellulose contents than those of the white variety, demonstrating its importance as dietary fibre (BACIC, 2003 [2]). In the Agrocycle European project [34] was reported that the main physicochemical characteristics of grape pomace are: crude protein range between 12.4-15.3, ash $(\%wt)^{db}$ as

2.7-7.8 (values are average values from a lot of samples), lignin (% wt)^{db} 33.3-52.8 (the "dry basis" (db) refers to the composition of the biomass excluding all water content).

A source of fatty acids, important for the feed industry, such as linolenic acid (which belongs to omega-3 fatty acids) was found for BMGM sample (0.31 g/100 g total FAME) and linoleic acids (belonging to the group of omega-6 fatty acids) for the MGM (63,54 g/100 g total FAME) and RIGM (61.39 g/100 g total FAME) samples (Table 2). It is known that GM results from the winemaking process were used as a natural antioxidant source because of the content in omega-3 PUFA for enriched diet formulations used for laying hens feed (Project EUREKA Veg4egg [26]).

Dried TRGM variety proved the highest amount of total amino acids compounds (8.5g/100g) compared to RIGM variety (7.7g/100 g), of which lysine (0.41g/100 g) and methionine (0.13 g/100 g) are the most important in nutrition (Table 3).

Table 2. Profile of the fatty acids (g/100 g total FAME) of the GM samples

SPECIFICATION		Fatty acids content (g/100g total FAME)			
		TRGM	RIGM	MGM	BMGM
Butiric	C4:0	0.07	0.04	0.00	0.06
Caproic	C6:0	0.15	0.16	0.14	0.19
Caprilic	C8:0	0.60	0.76	0.63	0.80
Capric	C10:0	0.44	0.54	0.49	0.57
Miristic	C14:0	0.80	1.03	0.77	0.98
Pentadecanoic	C15:0	0.04	0.11	0.02	0.10
Palmitic	C16:0	14.14	11.02	11.81	13.42
Palmitoleic	C16:1	0.32	0.46	0.42	0.53
Heptadecanoic	C17:0	0.12	0.10	0.08	0.11
Stearic	C18:0	3.67	4.03	3.83	4.17
Oleic cis	C18:1	16.09	16.94	15.34	17.35
Linoleic	C 18 : 2n6	57.84	61.39	63.54	57.84
Linolenic	C 18 : 3n6	0.21	0.06	0.23	0.31
Linolenic α	C 18 : 3n3	2.46	1.63	1.51	1.93
Octadecatetraenoic	C 18 : 4n30	0.76	0.60	0.00	0.00
Eicosadienoic	C 20(2n6)	0.17	0.15	0.37	0.05
Docosadienoic	C 22(2n6)	1.50	0.50	0.56	0.94
Docosatrienoic	C 22(4n6)	0.11	0.04	0.0	0.06
Eicosapentaenoic	C 20(5n3)	0.24	0.10	0.11	0.17
Lignoceric	C24:0	0.27	0.10	0.10	0.15
Nervonic	C 24 (1n9)	0.00	0.00	0.00	0.13
Other fatty acids		0.00	0.26	0.05	0.12

Table 3. The results of determining the GM samples amino acid content

Determined amino acids	Amino acid content (g/100g)			
	TRGM	RIGM	MGM	BMGM
Aspartic acid	1.10	0,90	0.98	1.03
Glutamic acid	2.0	1.49	1.79	1.86
Serine	0.49	0.55	0.53	0.47
Glycine	0.76	0.53	0.72	0.71
Treonine	0.57	0.58	0.55	0.66
Arginine	0.51	0.49	0.53	0.51
Alanine	0.44	0.48	0.49	0.52
Tirozine	0.22	0.15	0.19	0.24
Valine	0.49	0.49	0.16	0.49
Fenilalanina	0.44	0.45	0.44	0.40
Isoleucine	0.35	0.33	0.34	0.34
Leucine	0.69	0.58	0.60	0.59
Lysine	0.48	0.41	0.51	0.49
Cystine	n.d*	0.15	n.d*	n.d*
Methionine	n.d*	0.13	n.d*	n.d*
Total amino acids	8.50	7.7	8.2	8.30

The results of CASTRO SOUSA et al (2014) [7], demonstrated that grape by-products are an important source of nutrients and compounds with functional properties that suggest that they can be incorporated as an ingredient in the diet and/or used as a dietary supplement. VLAICU et al (2019) [33] used the by-products (flax

seed meal and grape seed meal), he demonstrated that this by-products rich in nutrients can be used in pigs feeding (with limits), which can improve the feeding quality of the pork meat. It is known that the seeds consist of 40% (w/w) fibre, 16% essential oil, 11% protein, 7% complex phenolic compounds, such as tannins, sugars, minerals and other substances (CAMPOS et al, 2008 [6]).

b. Spectroscopic analysis of dried GM samples

Fourier transform infrared spectroscopy (ATR-FTIR) was used in this study to qualitatively determine the chemical functional groups of GM samples. With the help of the peaks from the 1800-700 cm^{-1} spectral region the differences between the four analysed samples were detected; the characteristic functional groups absorb in the *fingerprint* region (BASALEKOU et al, 2015 [3]) (Table 4).

Figures 1, 2, 3 and 4 presented the spectral range ATR-FTIR obtained from the analysis of GMs powder, peaks at around 3275, 2922, 2848, 1732, 1607, 1521, 1435, 1304, 1263, 1047, and 782 cm^{-1} were clearly observed. Representative peaks observed in the selection region of 3309 to 3275 cm^{-1} is due to benzene nucleus and methyl tannin groups corresponds to the elongation -OH vibrations determined by phenolic compounds (tannins) present in all samples analysed (ALARA et al, 2018 [1], ZHANG et al, 2013 [35]). GM samples show representative peaks at 2922 cm^{-1} and 2849 cm^{-1} which is due to symmetrical and asymmetrical C–H stretch bonds in CH_2 and CH_3 groups, methyl bands links belonging to lipids (RICCI et al, 2015 [27]). The peaks were more intense in case of RIGM variety due to a large amount of seeds (FERREIRAA et al, 2014 [13]) (Figure 2).

Table 4. Wavenumber assignments and highest molar absorptivity (λ_{max}) of FTIR spectra of dried GM samples

Wavenumber (cm^{-1}) and λ_{max}								**Assignments**
TRGM		RIGM		MGM		BMGM		
3309	0.409	3310	0.139	3309	0.290	3310	0.349	–OH stretch vibration, -H bonded
3279	0.398			3275	0.268	3278	0.337	(νOH=3200-3600 cm^{-1})
2922	0.251	2915	0.458	2917	0.512	2917	0.251	C–H stretching, aliphatic
2852	0.193	2849	0.383	2849	0.401	2849	0.203	(2950-2800 cm^{-1})
1732	0.097	1732	0.071	1732	0.104	1715	0.068	Stretching vibration of C=O
		1684	0.096	1687	0.138			
1607	0.142	1607	0.092	1607	0,147	1606	0.127	romatic H bonds (OO)
1524	0.091	1521	0.066					C = C stretching bands
1433	0.115			1435	0.131	1435	0.106	Antisymmetric in-plane bending of $–CH_3$
1339	0.103	1317	0.067	1304	0.144	1307	0.105	Symmetric in-plane bending of $–CH_3$
1258	0.105			1263	0.149	1259	0.107	Stretching vibration of C–O
1212	0.092			1210	0.142	1211	0.098	
1050	0.199			1049	0.184	1047	0.199	Stretching vibration of C–O
1029	0.201	1031	0.087			1027	0.206	
781	0.020	719	0.030	788	0.033	777	0.017	CH out of-plane conformations

Several peaks occur in the region between 1800 to 700 cm^{-1}, representing the region of *fingerprints* significant for qualitative analysis, corresponding to the vibration of the C-O, C-C, C-H and C-N links. In this region it exhibits a distinctive peak in the 1732 cm^{-1} range for carbonyl stretching of hydrolyzable tannins (JENSEN et al, 2008 [17]), but with a weak signal, higher absorbance values are observed for the MGM variety (Figure 3). Signals in the 1684-1698 regions generally indicate the presence of flavonoids for the RIGM variety, or oxidation of some OH groups in flavonol molecules (KANNAN et al, 2011 [18]) (Figure 2).

Bands at 1607 and 1521 cm^{-1} were assigned to aromatic CH bonds and C=C stretch bands, respectively (FU et al, 2015 [15]), typical of TMGM variety aromatic molecules (Figure 1), which suggested the presence of polyphenols, flavonoids and amino acids. The peak at 1435 cm^{-1} was corresponding to antisymmetric in-plane bending of $–CH_3$ and peak at 1304 cm^{-1} was assigned with symmetric in-plane bending of –CH3 (ZHAO et al,

2015 [36]). The band of about 1263 cm^{-1} is characteristic of flavonoid-based tannins (DE SOUZA et al, 2015 [8]) while, the band of around 1027 cm^{-1} corresponds to phenolic compounds and sugar monomers (SAHA et al, 2016 [28]). Finally, the lower bands are related to CH bonds in aromatic structures (SARDELLA et al, 2015 [29]), the band at 788 cm^{-1} was assigned to the respiration form of the aromatic ring (ZHANG et al, 2013 [35]).

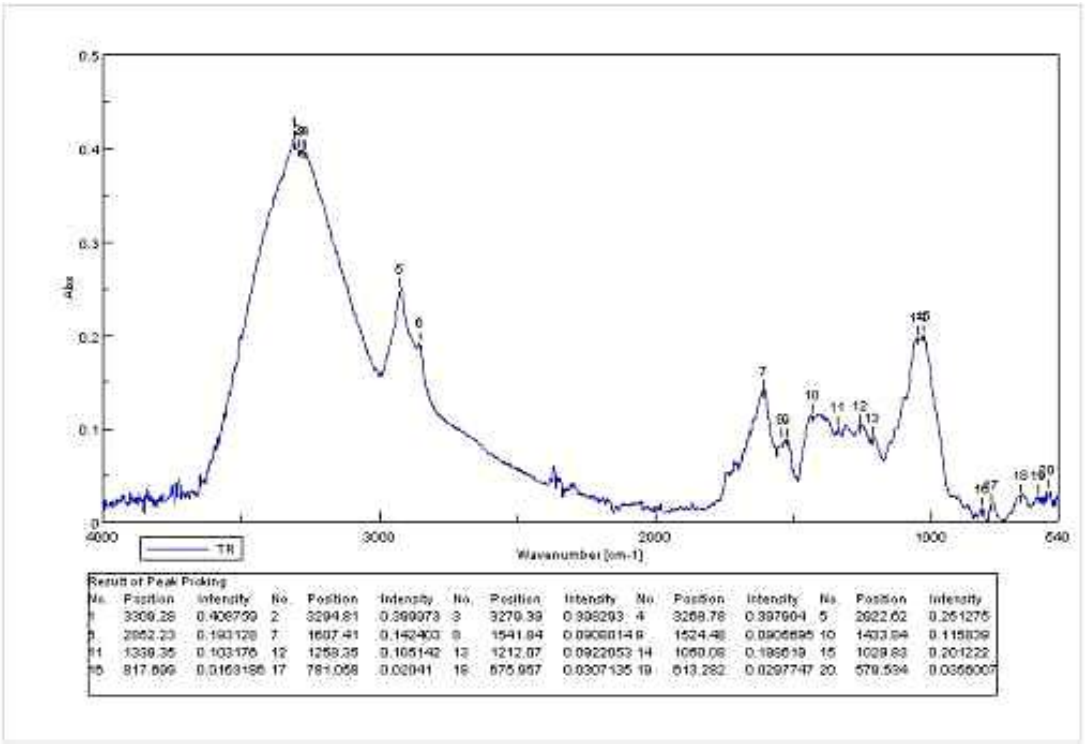


Figure 1. ATR-FTIR spectral analysis of the TRGM sample

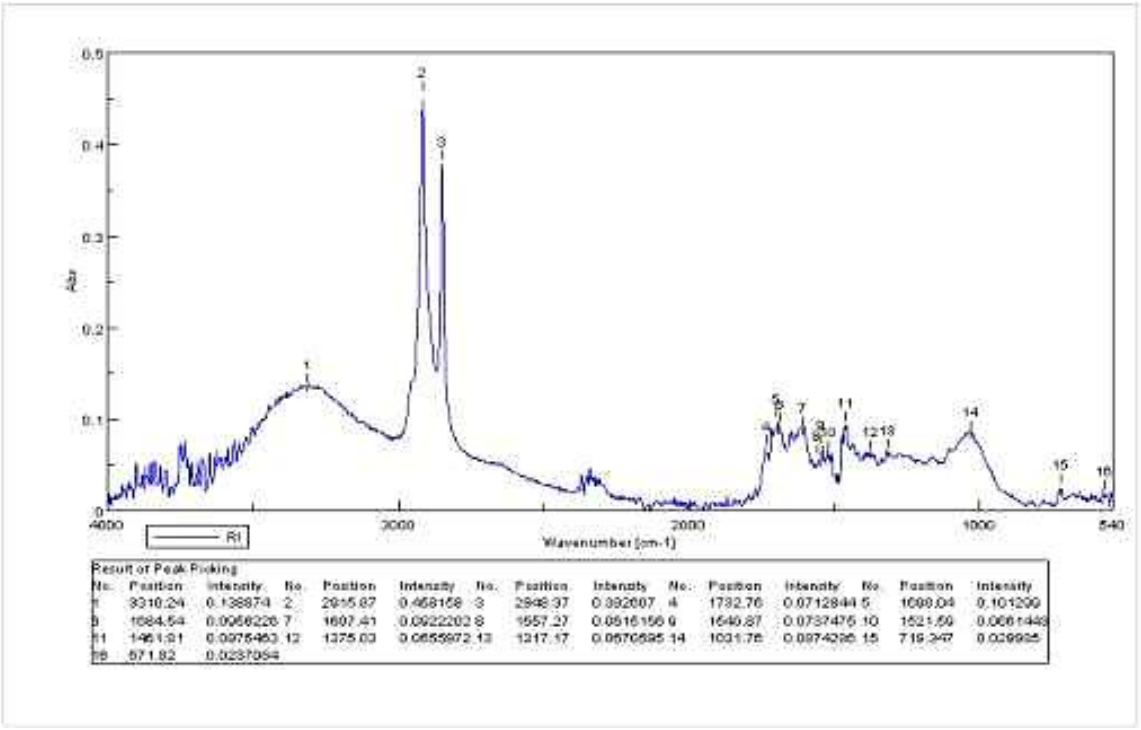
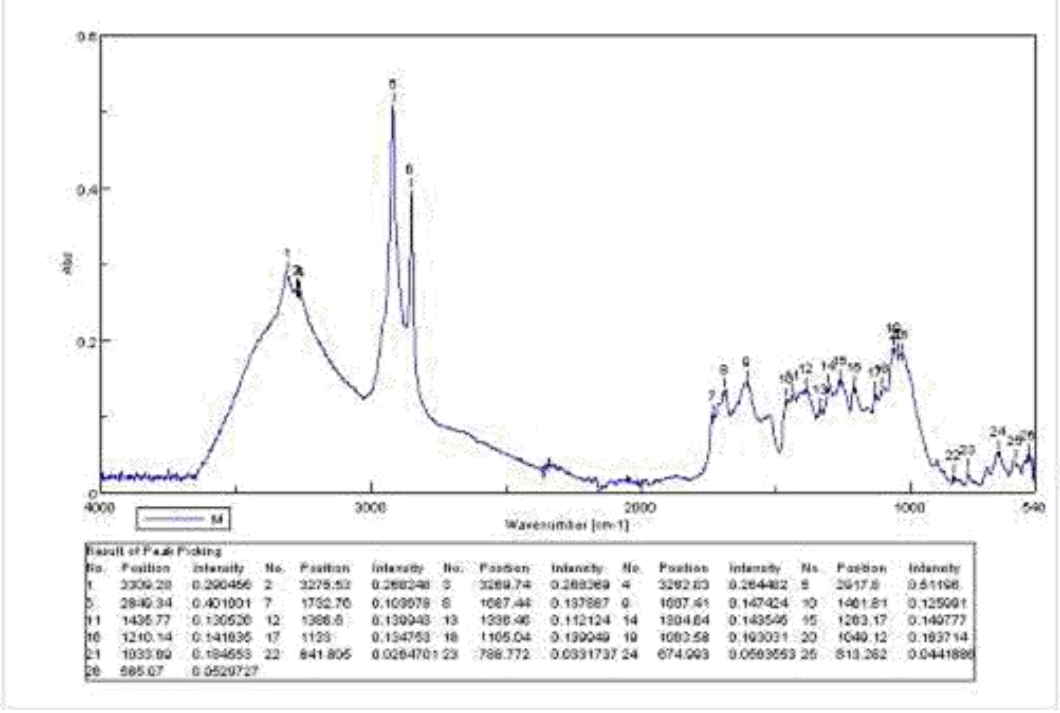


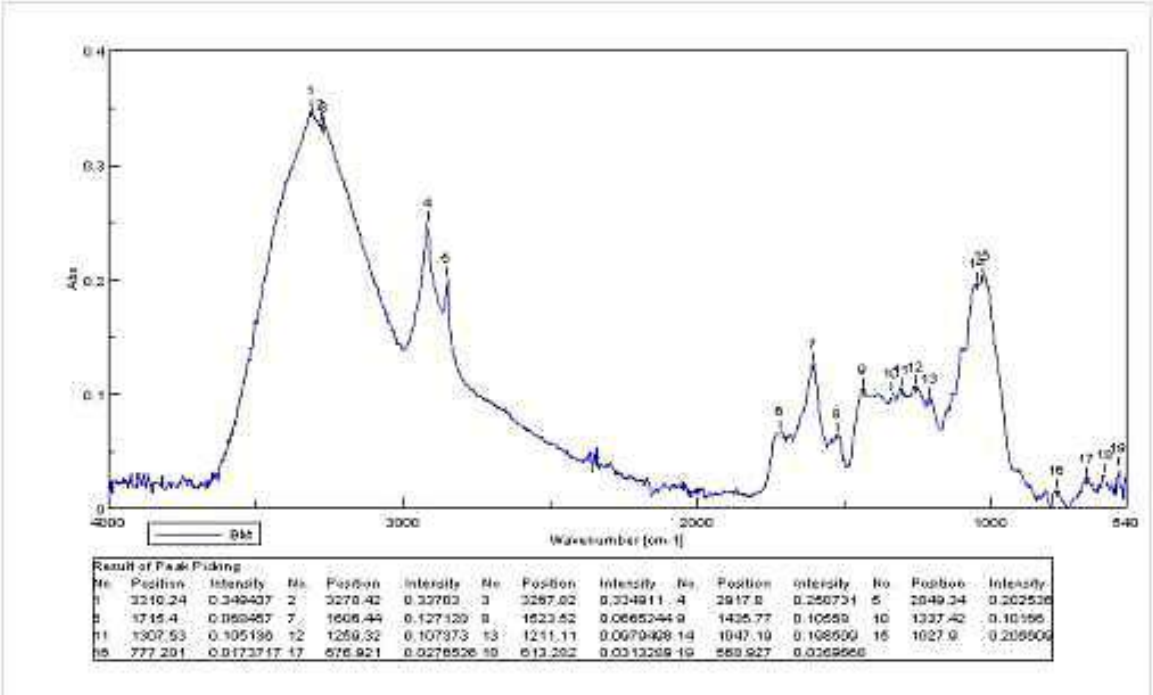
Figure 2. ATR-FTIR spectral analysis of the RIGM sample



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3309.28	0.280456	2	3275.53	0.288248	3	3289.74	0.288369	4	3282.03	0.284482	5	2917.8	0.51198
6	2849.34	0.401001	7	1732.76	0.109978	8	1667.44	0.137887	9	1607.41	0.147424	10	1461.61	0.125091
11	1435.77	0.130526	12	1396.6	0.139943	13	1336.46	0.112124	14	1304.64	0.143546	15	1263.17	0.149777
16	1210.14	0.141935	17	1123	0.134753	18	1105.04	0.139049	19	1093.58	0.193031	20	1049.12	0.193714
21	1033.69	0.184653	22	841.805	0.0264701	23	788.772	0.0331737	24	674.993	0.0569353	25	613.262	0.0441886
26	585.07	0.0529727												

Figure 3. ATR-FTIR spectral analysis of the MGM sample



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3310.24	0.349437	2	3276.42	0.33763	3	3287.02	0.334911	4	2917.8	0.256731	5	2849.24	0.202536
6	1715.4	0.069457	7	1606.44	0.127139	8	1523.52	0.0665244	9	1435.77	0.10559	10	1337.42	0.10156
11	1307.53	0.105186	12	1259.32	0.107373	13	1211.11	0.0979408	14	1047.19	0.108509	15	1027.9	0.208509
16	777.291	0.0173717	17	676.921	0.0276526	18	613.262	0.0313269	19	569.927	0.0269568			

Figure 4. ATR-FTIR spectral analysis of the BMGM sample

Conclusions

Sample of dried MGM variety proved a highest amount of crude protein (9.6%), crude lipids (8.5%) and cellulose (26.0%). Dried TRGM sample demonstrated the elevated content of total amino acids compounds (8.5 g/100 g) compared to variety of RIGM (7.7 g/100 g), from which methionine (0.13 g/100 g) and lysine (0.41 g/100 g) are the most significant in animal nutrition.

Linolenic acid (which belongs to omega-3 fatty acids) was found for dried BMGM sampe (0.31 g/100 g total FAME) and linoleic acids (belonging to the group of omega-6 fatty acids) for the dried MGM (63.54 g/100 g total FAME) and RIGM (61.39 g/100 g total FAME) samples.

FTIR analysis results showed that the composition of the chemical groups of the GMs will allow the integral use in feed. The by-products of the winemaking industry contain antioxidant compounds, polyphenols (anthocyanidins) from the grape skin, sugar, tannin, fibers, essential oils resulting from seeds, minerals and other substances, which is demonstrated by the peaks of the bands shown in the infrared spectrum. RIGM and MGM samples have in the band 2950-2800 cm^{-1} two very sharp peaks that demonstrate the high content of essential oils due to the presence of the seeds. The physicochemical and functional properties of GM as a functional ingredient will be further investigated.

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References

1. ALARA O.H., ABDURAHMAN N.H., UKAEGBU C.I., AZHARI N.H. *Vernonia cinerea* leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique, *Ind. Crops Prod.* 2018; 122, 533-544; https://doi.org/10.1016/j.indcrop.2018.06.034
2. BACIC T. Recovery of valuable products from lees and integrated approach to minimise waste and add value to wine production, *Grape and Wine Res. and Dev. Corp.* 2003; Project Number: UM 01/01.
3. BASALEKOU M., PAPPAS C., KOTSERIDIS Y., STRATARIDAKI A., GENIATAKIS E., TARANTILIS P.A., KALLITHRAKA S. Monitoring wine aging with Fourier transform infrared spectroscopy (FT-IR). BIO Web of Conferences, vol. 5(2015) 38th World Congress of Vine and Wine. https://doi.org/10.1051/bioconf/20150502016
4. BEKHIT A.E.A., CHENG V.J., HARRISON R., YE ZHIJING, BEKHIT A.A., NG T.B., LINGMING K. Technological aspects of by-product utilization. In: Bordiga, M. (Ed.), Valorization of wine making by-products. CRC Press. 2016, 117-198.
5. BRAHIM M., GAMBIER F., BROSSE N. Optimization of polyphenols extraction from grape residues in water medium, *Ind. Crop. Prod.* 2014; 52 (2014), 18-22. https://doi.org/10.1016/j.indcrop.2013.10.0300
6. CAMPOS L.M.A.S., LEIMANN F.V., PEDROSA R.C., FERREIRA S.R.S. Free radical scavenging of grape pomace extracts from Cabernet Sauvingnon (*Vitis vinifera*), *Bioresour. Technol*. 2008; 99(2008) 8413-8420; https://doi.org/10.1016/j.biortech.2008.02.058
7. CASTRO SOUSA E., UCHÔA THOMAZ A.M.A., BESERRA CARIOCA J.O., DE MORAIS S.M. et al. Chemical composition and bioactive compounds of grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semiarid region of Northeast Brazil, 135 *Food Sci. Technol, Campinas*, 2014; 34(1): 135-142, https://doi.org/10.1590/S0101-20612014000100020
8. DE SOUZA V.B., THOMAZINI M., BALIEIRO J.C.C., FÁVARO-TRINDADE C.S. Effect of spray drying on the physicochemical properties and color stability of the powdered pigment obtained from vinification byproducts of the Bordo grape (*Vitis labrusca*). *Food Bioprod. Process* 2015; 93 (2015), 39-50.
9. DENG Q., PENNER M.H. and ZHAO Y. Chemical composition of dietary fiber andpolyphenols of five different varieties of wine grape pomace skins. *Food Research International*. 2011; 44(2011), 2712-2720.
10. EL BOUSHY, A.R.Y. and VAN DER POEL A.F.B. Handbook of poultry feed from waste: processing and use. Springer-Verlag New York, p. 428.
11. ELKAHOUI S., DAO L., TAKEOKA G. Phenolic composition of grape pomace skin of four grape cultivars, *International Journal of Food Science and Nutrition*. 2018, vol. 3(6); 246-249.
12. FAO, 2016. FAOSTAT. Food and Agriculture Organization of the United Nations, Rome, Italy, 2016.
13. FERREIRAA A.S., NUNESA C., CASTROB A., FERREIRAB P., COIMBRA M.A. Influence of grape pomace extract incorporation on chitosan films properties; *Carbohydrate Polymers*: 2014; Vol. 113, (26) (2014), pages 490-499; https://doi.org/10.1016/j.carbpol.2014.07.032
14. FÉVRIER et al, 2009. In: MOLETTA R. Le traitement des déchets, Lavoisier, Editions Tech & Doc. 2009.
15. FU C.L., YANG X.N., LAI S.J., LIU C., HUANG S., YANG H. Structure, antioxidant and α-amylase inhibitory activities of longan pericarp proanthocyanidins, *J. Funct. Foods*, 14 (2015) 23-32; https://doi.org/10.1016/j.jff.2015.01.041
16. http://www.fao.org, F.-F.S.D., FAOSTAT-FAO Statistical Database, 2010.

17. JENSEN J.S., EGEBO M., MEYER A.S. Identification of spectral regions for the quantification of red wine tannins with Fourier transform mid-infrared spectroscopy. *J. Agr. Food Chem.*, 2008, 56: 3493-3499.
18. KANNAN R.R.R., ARUMUGAM R., ANANTHARAMAN P. Fourier transform infrared spectroscopy analysis of seagrass polyphenols. *Curr. Bioact. Compd.* 2011, 7: 118-125.
19. KARNOPP A.R., MARGRAF T., MACIEL L.G., SANTOS J.S. and GRANATO D. Chemical composition, nutritional and *in vitro* functional properties of byproducts from the Brazilian organic grape juice industry, *International Food Research Journal*. 2017, 24(1): 207-214 (2017).
20. MARIN S., BĂRBULESCU I.D., PERTEA A.M., MATEI P.M., MARINESCU S.I., TEODORESCU R.F., MARIN D., DUMITRACHE C., TUDOR V., TEODORESCU R.I. Valorisation of GM from Pietroasa vineyard for obtaining new dry feed ingredients as a potential polyphenols source, *Scientific Papers. Series B, Horticulture*. Vol. LXIII, No. (1), 2019 Online ISSN 2286-1580.
21. MENDES J.A.S., PROZIL S.O., EVTUGUIN D.V., LOPES L.P.C. Towards comprehensive utilization of winemaking residues: characterization of grape skins from red grape pomaces of variety Touriga, *Nacional. Ind. Crop Prod*. 2013. 43, 25-32.
22. MOATE P.J., WILLIAMS S.R.O., TOROK V.A., HANNAH M.C., RIBAUX B.E., TAVENDALE M.H., ECKARD R.J., JACOBS, J.L., AULDIST M.J., WALES W.J. 2014. GM reduces methane emissions when fed to dairy cows. *J. Dairy Sci.* 2014, 97 (8): 5073-5087 https://www.feedipedia.org/node/691
23. MOVASAGHI Z., REHMAN S., REHMAN I.U. Raman Spectroscopy of Biological Tissues; *Appl. Spectrosc. Reviews*, 2007, 42, 493-541 (2007).
24. NERANTZIS E.T. and TATARIDIS P. Integrated Enology-Utilization of winery by-products into high added value products. *e-J. Sci. Technol.* 2006, 1, 79-89.
25. Official Journal of the European Union 26.02.2009 EN L45/1 and AOAC Official Methods of Analysis (2005).
26. Project EUREKA Veg4egg, Valorization of wine processing by-products as natural antioxidants for the layer diets rich in polyunsaturated fatty acids), https://www.agricola.ro/en/veg4egg-2/
27. RICCI A., OLEJAR K.J., PARPINELLO G.P., KILMARTIN P.A., VERSARI A. Application of Fourier Transform Infrared (FTIR) Spectroscopy in the Characterization of Tannins. *Appl. Spectrosc. Rev.* 2015; 50, 407-442.
28. SAHA S., KURADE M.B., EL-DALATONY M.M., CHATTERJEE P.K., LEE D.S., JEON B.H. Improving bioavailability of fruit wastes using organic acid: An exploratory study of biomass pretreatment for fermentation. *Energy Convers. Manage*, 2016; 127, 256-264.
29. SARDELLA F., GIMENEZ M., NAVAS C., MORANDI C., DEIANA C., SAPANG K. Conversion of viticultural industry wastes into activated carbons for removal of lead and cadmium. *J. Environ. Chem. Eng*. 2015; 3 (1), 253-260.
30. SCHIEBER A., STINTZING F.C., & CARLE R. By-products of plant food processing asa source of functional compounds – recent developments. *Trends in Food Science & Technology*. 2001; 12, 401-413.
31. SPIGNO, G., MARINONI, L., GARRIDO, G.D. State of the Art in Grape Processing By-Products, Handbook of Grape Processing By-Products. 2017; http://dx.doi.org/10.1016/B978-0-12-809870-7.00001-6
32. TSENG A. and ZHAO Y. Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. *Food Chemistry*. 2013, 138(1): 356-365.
33. VLAICU P.A., PANAITE T.D., CORNESCU M.G., ROPOTA M., OLTEANU M., DRĂGOTOIU D. The influence of by-products on the production parameters and nutrient digestibility in fattening pigs diet (60-100 kg), *AgroLife Scientific Journal* – 8 (1) 2019, pp. 261-269, 2019ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718.
34. www.AgroCycle.eu – Agrocycle for a circular economy.
35. ZHANG Z.F., LV G., HE W., SHI L., PAN H., FAN L. Effects of extraction methods on the antioxidant activities of polysaccharides obtained from *Flammulina velutipes*, *Carbohydrate Polymers*. 2013; 98(2):1524-1531, http://dx.doi.org/10.1016/j.carbpol.2013.07.052
36. ZHAO X., ZHU H., ZHANG G. and TANG W. Effect of superfine grinding on the physicochemical properties and antioxidant activity of red grape pomace powders. *Powder Technology*. 2015; Vol. 286, 838-844; https://doi.org/10.1016/j.powtec.2015.09.025