Characterization of some Winery by-Products Used in Animal Feeds

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Abstract

Secondary products from winemaking are considered to be a rich source of bioactive compounds, mainly phenolic compounds, which can offer numerous opportunities to improve feed quality. This paper aims to characterize three winery by-products (grape marc, grape seed meal and grape seed oil) with regard to: the primary chemical composition; polyphenol content; antioxidant capacity; fatty acid profile and amino acid profile. Grape marc had 10.43% crude protein, significantly lower (P<0.05) compared to grape seed meal (12.70%). The polyphenols content was significantly different (P<0.05) between the tested raw materials, the highest concentration being found in the grape seed meal (28.05 mg EAG/g) and the smallest in the grape seed oil (0.28 mg EAG/g). Consequently, the antioxidant capacity significantly differentiated (P<0.05) among all raw materials, the highest value of 145.83 mM Trolox / g being recorded at grape seed meal, followed by 9.44 mM Trolox / g in grape marc and 0.70 Mm Trolox / g to grape seed oil. The highest lysine content (0.58 g/100 g SU) was recorded in the marc, while the cystine (0.24 g/100 g SU) was recorded in grape seed grains. The obtained results show that these winery by-products can be used as natural antioxidants in animal feed.

Keywords: animal feed, natural antioxidant, winery by-products

1. Introduction

Nutrition plays an important role in maintaining animal health and is also the main factor affecting the quality of products derived from them. An insufficient intake of antioxidants or a high pro-oxidant intake can lead to oxidative stress [1]. Animal nutrition is currently focusing on omega-3 polyunsaturated fatty acids (PUFAs) to improve the quality of animal products [2-4], but this nutritional strategy has been associated with an increase in lipoperoxidation in subcutaneous and intramuscular lipids [5].

The wine industry produces huge quantities of by-products (skin, seeds, meal, etc.) containing a wide range of bioactive compounds [6], such as polyphenols and flavonoids, and which can offer many benefits due to the properties they own. Using these by-products as natural antioxidants in diets could improve the quality of feed and, implicitly, the animal products quality by the oxidative stability conferred [7]. Recovery of antioxidant compounds from these residues is also important for maintaining the environment's balance, as large quantities of residues are generated in wine-growing areas, which is an ecological and economical problem of storage, transformation or disposal [8].

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This situation explains the increased interest in the use of winemaking by-products as a rich and inexpensive source of polyphenols [9-10]. In vivo and in vitro conducted studies in the recent years have demonstrated the beneficial effects of administering these bioactive compounds in monogastric animals diets due to the antioxidant, antimicrobial and anti-inflammatory activity they exert [11-15]. It is noteworthy that most reports on the beneficial effects of polyphenols have been obtained from in vitro studies. Although the researchers have shown an increased interest in the properties of the by-products, these sources of antioxidants have been insufficiently exploited so far, the information being therefore limited [16].

The purpose of this paper is to evaluate the main nutrient content of some winery by-products and the potential to use them as natural sources of antioxidants in animal feed, with an important activity in the prevention of oxidative reactions.

2. Materials and methods

The winery by-products characterized in this study were: grape marc, grape seed meal seed and grape seed oil, purchased from wine producers and processing plants in South-Eastern Romania.

Grape marc is the total of the plant parts of the grapes, used to obtain the must or wine by the pressing process. It is composed of skins, seeds and bunches as well as other components [17] according to grape processing technology, accounting for about 20% of the weight of grapes converted to wine [18]. Grape seed meal is the by-product of the process of extracting seed oil by cold pressing or by chemical means. Seed grape is generally used to produce edible oil with a high nutritional value, by cold pressing.

In order to determine the nutritional quality of the tested by-products, primary chemical composition determinations were made on: dry matter, crude protein, ether extractives, cellulose, ash; polyphenol content; antioxidant capacity; fatty acid profile and amino acid profile.

These determinations were made using standardized methods in accordance with Regulation (CE) no. 152/2009–Methods of sampling and analysis for the official inspection of feeds.

Dry matter (DM) was determined by the gravimetric method using BMT drying oven model ECOCELL Blueline Comfort model (Nuremberg, Germany); the crude protein (CP) was determined by the Kjeldahl method using a semiautomatic system KJELTEC auto 2300-Tecator (Sweden); the ether extractives (EE) was determined by the organic solvent extraction method using a SOXTEC-2055 FOSS-Tecator system (Sweden); the ash (Ash) was determined by gravimetric method using Caloris CL 1206 furnace.

Gross energy (GE) was determined by calculation, using the gross chemical analysis data and the equations developed by [19].

The amino acids were determined by the liquid chromatographic method using a Finningan Surveyor Plus HPLC (Thermo-Electron Corporation, Waltham, USA), fitted with PDA detector (Photo Diode Array Detector). The amino acids are separated on a Hypersil BDS C18 column with silica gel (250×4.6 mm), particle size 5 μm, with reverse phase and a 45^{o}C temperature. The results were expressed in g amino acids/100 g DM.

The fatty acids were determined by gas chromatography, according to standard SR CEN ISO/TS 17764 -2: 2008, using Perkin Elmer-Clarus 500 gas chromatograph, with capillary column injection system, high polarity stationary phase (BPX70: 60mx0.25mm inner diameter and 0.25μm thick film), and high polarity cyanopril phase, which give similar resolution for different geometric isomers (THERMO TR-Fame: 120mx0.25mm IDx0.25μm film). The results were expressed in g acid/100 g fat.

The grape seed samples concentration of polyphenols was determined according to the method described by Mihailović et al. (2013) [20] using a Thermo Scientific UV-VIS spectrophotometer. The results were expressed in mg equivalent gallic acid/g sample (mg EAG/g sample).

The antioxidant capacity of the grape seed samples was determined according to the method proposed by Marxen et al. (2007) [21] using a UV-VIS Analytik Jena Specord 250 Plus Spectrophotometer. The results were expressed in mM Trolox equivalents/g sample (mM ET/g sample).

The analytical data were compared by variance analysis (ANOVA and t test), using StatView for Windows (SAS, version 6.0). The experimental results were expressed as mean values±standard error, the differences being considered statistically significant for P≤0.05.

3. Results and discussion

The effectiveness of natural antioxidants depends primarily on their chemical composition and dose included in feed formulations. The chemical composition of winery by-products depends, in turn, on the grape variety, the type of soil, the agro-climatic factors and the vinification techniques applied [9].

Table 1 shows the results of the primary chemical composition of tested by-products in this study. As it can be seen, grape seed meal has been highlighted by a significantly higher content (P≤0.05) of 12.70% crude protein, compared to grape marc which had 10.43% crude protein. Significantly higher differences (P≤0.05) for grape seed meal have also been recorded with regard to the ether extractives content (6.81%) and gross energy (18.38 MJ/kg), unlike the grape marc which had 4.87% ether extractives, respectively 15.90 MJ / kg of gross energy. The presented data in this paper regarding to the primary chemical composition of the grape seed meal are in agreement with those in the literature which reported a concentration of 97.4% dry matter, 9.3% crude protein, 10.9% ether extractives and 2.7% ash [22]. Also, a number of researches showed for grape meal a concentration ranging from 11.2-13.8% for crude protein [18, 23]; 5.6-11.7% ether extractives [23, 24]; 32.5 -56.3% cellulose [18, 23, 25]; respectively 2.4-5.8% ash [23, 24]. Also, researchers [26] studying grape meal, reported a concentration of 11.5% crude protein, 6.5% ether extractives and 8.1% ash. In another study regarding on the inclusion of grape meal in broiler diets, Brenes et al. [27] determined its primary chemical composition, reporting a concentration of 13.85% crude protein, 9.87% ether extractives, 15.18% cellulose and 2.41% ash. The results regarding on the content of the main nutrients of the grape marc presented in Table 1 show similar concentrations to those in the literature: 12.2% crude protein; 6.39% ether extractives and 86.6% organic matter [28].

Table 1. Content of the main nutrients and gross energy of the tested raw materials

Specification	Grape marc	Grape seed meal	SEM	P
Dry matter, %	84.45^a	91.35^b	1.404	0.0027
Organic matter, %	78.91^a	88.34^b	1.770	0.0002
Crude protein, %	10.43^a	12.70^b	0.537	0.0224
Ether extractives, %	4.87^a	6.81^b	0.401	0.0037
Cellulose, %	23.26^a	34.31^b	2.105	0.0004
Ash, %	5.54^a	3.00^b	0.457	<0.0001
Gross energy, MJ/kg	15.90^a	18.38^b	0.455	<0.0001

Where: a-b Mean values within a row having different superscripts are significantly different by least significant difference test (P≤0.05); SEM-standard error of the mean; means in the same row no common superscript significantly different (P≤0.05).

The recorded data for the determined amino acid profile in the tested by-products are shown in Table 2 as mean±standard error. The grape marc was characterized by 2.97 g/100 g DM content of essential amino acids, 1.07 g/100 g DM content of semi-essential amino acids, and 4.16 g/100 g DM content of non-essential amino acids.

Compared to the grape marc, the grape seed meal showed higher concentrations of amino acids, respectively a 4.18 g/100 g DM content of essential amino acids, 2.41 g/100 g DM content of semi-essential amino acids and 6.51 g/100 g DM content of non-essential amino acids.

The presented results are in agreement with those in the literature that evaluated the nutritional value and the antioxidant potential of secondary products from winemaking. Olteanu et al. [29] presented for grape marc a threonine content of 0.38 g/100 g DM, arginine 0.54 g/100 g DM, valine 0.42 g/100 g DM, phenylalanine 0.36 g/100 g DM, isoleucine 0.62 g/100 g DM, leucine 0.62 g/100 g DM, lysine 0.54 g/100 g DM and methionine 0.09 g/100 g DM.

In a study on the nutritional value of some by-products obtained from the food industry, the researchers [30] reported the following amino acid concentrations for grape seed meal: 0.63 g/100 g DM threonine, arginine 0.90 g/100 g DM, valine 0.64 g/100 g DM, phenylalanine 0.52 g/100 g DM, isoleucine 0.48 g/100 g DM, leucine 0.85 g/100 g DM, lysine 0.42 g/100 g DM and methionine 0.72 g/100 g DM.

Table 2. Amino acids profile of the tested raw materials, (g/100 g DM)

Specification	Grape marc	Grape seed meal
Aspartic acid	0.72±0.05	1.22±0.07
Glutamic acid	2.33±0.08	3.52±0.26
Serine	0.20±0.00	0.69±0.11
Glycine	0.60±0.03	1.08±0.22
Threonine	0.40±0.03	0.66±0.12
Arginine	0.53±0.02	1.19±0.17
Alanine	0.51±0.03	0.69±0.08
Tyrosine	0.19±0.01	0.29±0.04
Valine	0.44±0.03	0.70±0.09
Phenylalanine	0.38±0.02	0.63±0.08
Isoleucine	0.44±0.05	0.56±0.05
Leucine	0.64±0.03	0.95±0.10
Lysine	0.58±0.05	0.49±0.06
Cystine	0.15±0.00	0.24±0.03
Methionine	0.09±0.01	0.19±0.03

Winery by-products were also analysed for ether extractives content. In grape marc, the highest concentrations were found for linoleic acid (63.69 g/100 g fat), oleic acid (17.32 g/100 g fat), palmitic acid (11 g/100 g fat) and stearic acid (3.93 g/100 g fat). The results are consistent with those presented in the specialty studies which reported for grape marc a 58.99 g/100 g fat concentration of linoleic acid; 16.86 g/100 g of fat of oleic acid, 14.36 g/100 g fat of palmitic acid and 3.89 g/100 g fat of stearic acid [29].

Also, in the grape seed meal, linoleic acid has a rich content of 65.66 g/100 g of fat. The determined concentration of oleic acid was similar to that obtained in the grape marc, respectively 17.88 g/100 g fat, but the content of palmitic acid was lower, 8.95 g/100 g fat, as stearic acid (3.43 g 100 g fat). For grape seed meal, Correddu et al. [22] shows a 8.5 g/100 g fat concentration of palmitic acid; 4.9 g/100 g fat of stearic acid; 9.6 g/100 g fat of oleic acid; 74 g/100 g fat of linoleic acid and 0.3 g/100 g fat of linolenic acid.

Regarding the fatty acid profile of grape seed oil, it is well known that it contains predominantly polyunsaturated fatty acids (64-75%). The linoleic acid is the most abundant fatty acid, followed by oleic acid, stearic acid and palmitic acid in grape seed oil [31]. The data from this paper regarding on grape seed oil shows a 67.18 g/100 g fat concentration for linoleic acid, 18.61 g/100 g fat of oleic acid, 9.15 g/100 g fat of palmitic acid, and 3.52 g/100 g fat for stearic acid, that are similar to those obtained for grape seed oil by a number of researchers who reported a content ranging between 7.05-7.70 g/100 g fat of palmitic acid; 2.52-4.72 g/100 g fat of stearic acid; 13.9-21.9 g/100 g fat of oleic acid; 66-705.3 g/100 g fat of linoleic acid; 0.25-1.14 g/100 g fat of linolenic; respectively 0.15 g/100 g fat of arachidic acid [32].

Table 3 shows the results on the fatty acid content of raw material samples tested as function of the degree of unsaturation. The highest concentration of PUFA was identified in grape seed meal, with 68.80 g/100 g fat, followed closely by grape seed oil with a 68.17 g/100 g fat concentration and grape marc with 66.43 g/100 g fat. Higher concentrations of omega-3 fatty acids were determined, as can be seen both for the grape marc (1.91 g/100 g fat) and for the grape seed meal (1.75 g/100 g fat), those in the oil being lower in this study (0.72 g/100 g fat). In the omega-6 fatty acids case, higher concentrations were obtained in the grape seed meal (67.06 g/100 g fat) and grape seed oil (67.45 g/100 g fat), grape marc having a lower content this time. (64.51 g/100 g fat).

Table 3. Fatty acids content of the tested raw materials by level of unsaturation, (g/100 g fat)

Specification	**Grape marc**	**Grape seed meal**	**Grape seed oil**
SFA, %	16.94	14.44	12.97
MUFA, %	19.24	18.20	18.81
UFA, %	85.67	87.01	86.98
PUFA, % of which	66.43	68.80	68.17
Ω3, %	1.91	1.75	0.72
Ω6, %	64.51	67.06	67.45

SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; UFA- unsaturated fatty acids; PUFA- polyunsaturated fatty acids; Ω3- omega 3 polyunsaturated fatty acids; Ω6- omega 6 polyunsaturated fatty acids.

The presented data are similar to those obtained by Olteanu et al., [29] who reported for grape marc a concentration of 62.33% PUFA; 20.09% SFA, and 80.14% for UFA. Regarding the grape seed meal, other studies [30] revealed for the fatty acid profile the following concentrations: 64.71% PUFA; 1.47% omega-3; 63.23% omega-6; 13.48% SFA, 21.34% MUFA and respectively 86.05% UFA. Data for grape seed oil in literature [32] shows a concentration of 66.3-75.8% PUFA, 9.66-12.6% SFA; 14.5-22.2% MUFA.

Grape marc is a rich source of polyphenols, but their distribution in skin and seeds is different [33]. In the present study, the polyphenols concentration, respectively 3.03 mg EAG/ g (Table 4), was significantly ($P \leq 0.05$) lower than that determined in grape seed meal, respectively 28.05 mg EAG/ g polyphenols. Grape seed oil has the lowest content of polyphenols (0.28 mg EAG/ g), significantly different from the other two raw materials analysed.

Regarding the antioxidant capacity, significant differences ($P \leq 0.05$) were recorded among all three tested winery by-products (Table 4). Grape seed meal revealed a 145.83 mM ET/g, the highest value of antioxidant capacity, followed by grape marc which had a 9.44 mM ET/g. value. Grape seed oil has the lowest antioxidant capacity. The literature results are varied with regard to the polyphenols content of the by-products obtained from winemaking. In contrast to the reported data for grape marc in this study, the literature shows a higher content of polyphenols, ranging from 19 to 40.5 mg EAG/ g sample [34], or 20.2 mg EAG/g sample [35]. Regarding on grape seed meal, the presented data in Table 4 are similar to those recorded by other researchers, respectively 21.4 to 26.7 mg EAG/g sample [25] or 26.3 mg EAG/g sample [18]. There is a data series which showing higher concentrations compared to those obtained in this study grape seed meal, such as 48.3 mg EAG/g sample [36] and 34.9 mg EAG/ g sample [37]. Lutterodt et al. [32] reported a variable content of polyphenols ranging from 0.16 to 0.44 mg EAG/g for grape oil and 5.96 to 89.6 mg EAG/ g for grape meal, respectively antioxidant capacity values ranging from 0.07 to 2.22 mM ET/g for grape oil and 11.8 to 15 mM ET/g for grape meal.

Table 4. Content of polyphenols and antioxidant capacity of the tested raw materials

Specification	Grape marc	Grape seed meal	Grape seed oil	SEM	P
Polyphenols (mg EAG/g sample)	3.03^a	28.05^b	0.28^c	3.414	<0.0001
Antioxidant capacity (mM ET/g sample)	9.44^a	145.83^b	0.70^c	18.452	<0.0001

Where: a-c Mean values within a row having different superscripts are significantly different by least significant difference test ($P \leq 0.05$); SEM-standard error of the mean; means in the same row no common superscript significantly different ($P \leq 0.05$)

4. Conclusions

The by-products described in this paper demonstrate that they can be successfully used in animal feeds. Due to the high level of nutrients (proteins, amino acids, fatty acids) and their high antioxidant capacity, winery by-products are able to satisfy the requirements of the animal body. Of all the raw materials tested in this study, grape seed meal and grape seed oil were noted for a rich PUFA content, and grape seed meal revealed a high concentration of polyphenols and antioxidant capacity. Using these by-products as natural antioxidants in animal diets could improve the quality of feed and, implicitly, the quality of animal products by the oxidative stability that they confer.

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