

Nutritional Characterization of White Grape Pomace: Potential Feed Additive in Ruminants' Nutrition

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Abstract

In today's food industry, fortifying food products with several nutrients is a priority, and animal nutrition represents a key strategy for achieving this goal. Nevertheless, the scarcity and high costs of conventional feedstuffs lead to investigation of industrial by-products as alternative solutions in livestock nutrition. White grape pomace, a by-product of the winery industry, is well-known for its rich resveratrol content. Nevertheless, in addition to this, it can present significant quantities of other nutrients and antioxidant compounds, which exert beneficial effects when incorporated into ruminants' nutrition. Our study revealed a remarkable concentration of the white grape pomace nutrients, with important minerals, particularly manganese (106.35 mg/kg). The fatty acids profile showed a high composition of polyunsaturated fatty acids (65.05 g/100 g FAME), with a great amount of omega 3 fatty acids (62.66 g/100 g FAME). Concerning the antioxidant compounds, white grape pomace exhibited a concentration of 12.49 mg/g GAE for total polyphenols and 3.27 mg/kg for total flavonoids. Also, our study highlighted its high antioxidant potential, especially assessed through the DPPH radical scavenging assay (74.26 mM eq. Trolox), ABTS radical scavenging assay (75.07 mM eq. Trolox), and total antioxidant capacity (286.26 mM eq. ascorbic acid).

Keywords: Antioxidant potential, bioactive compounds, nutritional quality, white grape pomace

1. Introduction

A balanced diet plays a vital role in maintaining good health. With the world's population expanding at a fast rate, the food industry is faced with the challenge of meeting the growing demand for nutrient-rich animal products. One of the most important strategies aimed to enrich the animal food product with nutrients is represented by the animal nutrition. However, due to the high cost and limitations of feedstuffs, it is becoming increasingly important to explore alternative feed options [1].

The wine production industry generates a significant amount of waste residues, up to 16

million tons. This represents a high amount, considering that more than 30% of the grapes used for wine production end up as winery byproducts [2]. These byproducts, mainly grape pomaces, and stems, contain substantial amounts of physiologically active substances, such as polyphenols, that have positive health effects. While research was mainly focused on pomace from red grape varieties, there has been limited exploration of those from white varieties, which could also present a diverse range of potentially bioactive compounds [2, 3]. The quality and quantity of grape pomace polyphenols can vary significantly, depending on factors such as grape varieties, cultivation locations, winemaking techniques, and even geographical regions [4-6]. Grape skin and seeds are the primary sources of polyphenols in grape pomace, which are divided into two main categories: flavonoids (including anthocyanins and flavanols) and non-flavonoids

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(such as phenolic acids and tannins) [7]. These compounds have various biological activities and are linked to health benefits such as preventing cancer, protecting against microbial infections, and promoting cardiovascular and metabolism health [8]. Furthermore, besides polyphenols, grape pomace can represent a rich source of other bioactive substances such as minerals, vitamins, organic acids, and carbohydrates, which are important for human health [9].

According to several studies, grape pomace contains a moderate to high concentration of total protein (9-15% DM) and has great potential to be used in the diets of ruminants [10-11]. However, it is recommended that the inclusion level of grape pomace should not exceed 30% due to its high content of lignin and polyphenolic compounds [12]. Studies have shown that including polyphenols (especially tannins) in the diets of ruminants can lead to an improvement in nitrogen utilization and contribute to better growth performance [11]. Furthermore, the bioactive compounds presented in grape pomace can also enhance the quality of milk and meat [13, 14]. Since grape pomace is generally considered waste in the winery industry and remains unexploited, its inclusion in the diets of ruminants has the potential to reduce feed costs [15].

In this context, this study aimed to nutritionally characterize the white grape pomace and to determine its antioxidant capacity, considering its potential application in ruminants' nutrition.

2. Materials and methods

The studied white grape pomace was purchased from a local market (Bucharest, Romania).

The Kjeldahl method with the Kjeltac auto 1030 Tecator System (Höganäs, Sweden) was used to determine the total crude protein concentration, while the crude fat content was obtained using the Soxhlet method with the Soxtec 2055 Foss Tecator (Höganäs, Sweden). The total ash content was obtained using a gravimetric method and an ashing furnace (Nabertherm GmbH, Lilienthal, Germany) [16].

The trace mineral content, including copper, iron, manganese, and zinc, was measured using flame atomic absorption spectrometry after a microwave digestion step performed with a Thermo Electron

SOLAAR M6 Dual Zeeman Comfort System. (Cambridge, UK) [17].

The fatty acid profile was determined using the gas chromatographic method, with a Perkin Elmer Clarus 500 gas chromatograph (Massachusetts, United States) and a TRACE TR-Fame column (Thermo Electron, Massachusetts, United States). The column's stationary phase was made of high-polarity material. The fatty acid identification and quantification were performed using referencing standards, and detection was done using a flame ionization detector (FID) [18].

The determination of the vitamin E isomers was performed using high-performance liquid chromatography (HPLC), with a Vanquish Core Thermo-Electron System (Waltham, MA, USA), using an Accucore C18 column with follows dimensions: 4 µm particle size, 150 x 4.6 mm. The mobile phase consisted of water (4%) and methanol (96%), and the flow rate was 1.5 ml/min [16].

The total polyphenols content (TP) of the white grape pomace was assessed through the Folin-Ciocalteu method, as presented by [19]. The results obtained were expressed as mg of gallic acid equivalents / g (dried sample).

The total flavonoid determination was studied using the method presented by [20], involving the colorimetric method with aluminium chloride. The results were expressed as mg of quercetin equivalents/g.

The antioxidant capacity of the white grape pomace was studied using four methods, ABTS, DPPH, iron chelating capacity and total antioxidant capacity. The ability of the studied pomace to scavenge the 2,2'-azino-bis (3-ethylbenzothiazoline-6- sulfonic acid) radical (ABTS) was determined using the method described by [16], with a Jasco V-530 spectrophotometer (Japan Servo Co.Ltd., Japan).

The determination of the antioxidant capacity by the 2,2-diphenyl-1-picryl-hydrazyl-hydrate method (DPPH) was studied using a Jasco V-530 spectrophotometer (Japan Servo Co.Ltd., Japan), and the results were expressed as mmol Trolox equivalents per kg of sample [21].

The iron chelating capacity was performed by spectrophotometric method, using a Jasco V-530 spectrophotometer (Japan Servo Co.Ltd., Japan), by determining the samples' absorbances at 562 nm. The results were expressed as mg of the

ethylenediamine tetra-acetic acid equivalents (EDTA) / g (samples) [16].

The determination of the total antioxidant capacity (TAC) was performed using the phosphomolybdenum method, by determining the sample absorbances at 695 nm. The results were expressed as equivalents of ascorbic acid [16].

For each method assessed in the present work, we analysed samples in triplicate (n=3). The graphs were generated using Prism-GraphPad software (San Diego, CA, USA).

The Pearson correlation presented as a heatmap between the fatty acids' composition, antioxidant compounds, and antioxidant potential for the white grape pomace was obtained using Prism-GraphPad software (San Diego, CA, USA). The yellow colours correspond to the positive correlation coefficients, while the dark blue

colours correspond to negative correlation coefficients.

3. Results and discussion

The proximate chemical composition of the white grape pomace is presented in Table 1.

The results of our study showed that the composition of dry matter, total protein, total fat, and cellulose aligns with the values reported in the literature. The protein content was found to be in the range of 3.57-14.17 g/100 g, the total fat content in the range of 1.14-13.90 g/100 g, and the dietary fibres in the range of 17.28-88.70 g/100 g. Additionally, the dry matter contained approximately 907-966 g/kg [22-24].

Table 1. The proximate chemical composition of the white grape pomace

Parameters (%)	WGP ¹
Dry Matter	96.37 ±1.102
Total Protein	9.27±1.025
Total Fat	7.28±0.985
Ash	15.53±1.025
Cellulose	18.80±0.786

¹ White Grape Pomace

In our study, the content of total ash obtained was higher than the data reported in the literature. This could be explained by the fact that the proximate composition of grape pomace significantly depends on factors such as grape variety,

cultivation conditions, or processing techniques [22]. From the perspective of the chemical composition of white grape pomace, the trace minerals are also important components, and the results are presented in Figure 1.

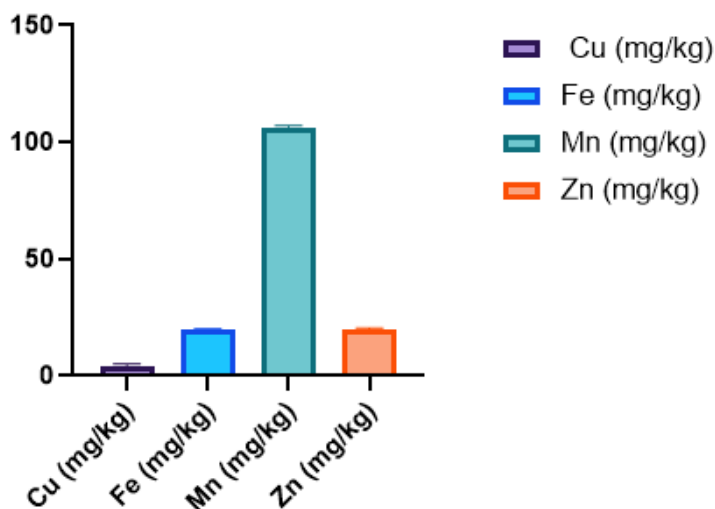


Figure 1. Trace minerals composition of the white grape pomace

According to our research, white grape pomace contains a significant amount of manganese, which is the main trace mineral present in its composition. However, the literature suggests that iron and zinc are the main minerals found in grape pomace. The variation in mineral composition can be influenced by environmental conditions, soil, and grape variety. Therefore, there is a high degree of variability in the mineral composition of grape pomace [22].

The fatty acid profile of the white grape pomace was evaluated, and the results are presented in Table 2. In our study, eighteen fatty acids were detected. The major fatty acids identified in the studied white grape pomace were the palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2n6), and alpha-linolenic acid (C 18:3n3), data

according with results presented in other studies [23]. The unsaturated fatty acids comprised the largest proportion of the white grape pomace fatty acids (82.05%), with polyunsaturated fatty acids (PUFA) being the major compounds. The analysis also revealed a higher concentration of omega fatty acids, specifically omega 6. According to the literature, it is important to supplement the diets of ruminants with natural sources of essential fatty acids. Linoleic acid and alpha-linolenic acid are the most abundant polyunsaturated fatty acids found in white grape pomace. They can be converted to dihomo- γ -linolenic acid, arachidonic acid, and eicosapentaenoic acid (EPA), which are involved in the synthesis of prostaglandins and many physiological processes [25]. In terms of monounsaturated fatty acids (MUFA), oleic acid was the most abundant.

Table 2. Fatty acids profile for the white grape pomace

	gFAME ¹ /100g	WGP ²
Caprylic acid	C 8:0	0.15±0.013
Capric acid	C 10:0	0.47±0.013
Lauric acid	C 12:0	0.07±0.005
Myristic acid	C 14:0	1.45±0.055
Myristoleic acid	C 14:1	0.36±0.003
Pentadecanoic acid	C 15:0	0.05±0.004
Pentadecenoic acid	C 15:1	0.04±0.004
Palmitic acid	C 16:0	11.46±0.042
Palmitoleic acid	C 16:1	1.51±0.02
Heptadecanoic acid	C 17:0	0.06±0.009
Heptadecenoic acid	C 17:1	0.05±0.052
Stearic acid	C 18:0	3.62±0.042
Oleic acid	C 18:1	15.02±0.043
Linoleic acid	C 18:2n6	61.31±0.031
Alpha-linolenic acid	C 18:3n3	2.39±0.051
Eicosadienoic acid	C 20:2n6	0.75±0.062
Eicosatrienoic acid	C 20:3n6	0.21±0.006
Docosadienoic acid	C22 (2n6)	0.37±0.0780
	Other Fatty acids	0.67±0.005
	ΣSFA ³	17.28±0.107
	ΣMUFA ⁴	17.00±0.003
	ΣPUFA ⁵	65.05±0.104
	ΣUFA ⁶	82.05±0.102
	Omega 3	2.39±0.052
	Omega 6	62.66±0.053
	Ω6/Ω3 ⁷	26.21±0.546

¹ Fatty acid methyl esters, ² White grape pomace, ³Total of saturated fatty acids, ⁴Total of monounsaturated fatty acids, ⁵Total of polyunsaturated fatty acids, ⁶Total of unsaturated fatty acids, ⁷ Ratio between omega 6 and omega 3 fatty acids.

This type of MUFA plays a crucial role in the nutrition of ruminants. According to the literature, increasing its concentration in milk can lead to decreases in levels of plasma cholesterol, LDL-cholesterol, and triacylglycerol concentrations. This, in turn, may reduce the risk of developing coronary artery disease for consumers [26]. Moreover, white grape pomace contains lower levels of medium-chain fatty acids C8-C12 (MCFA) which are essential for mammal energy metabolism and anabolic processes [27]. After studying the high content of unsaturated

fatty acids that are susceptible to oxidation, we analysed the antioxidant compounds presented in white grape pomace, including the vitamin E isomers. The results are presented in Table 3. White grape pomace contains a significant amount of total vitamin E, mainly in the form of alpha-tocopherol, followed by gamma-tocopherol, which is according to the observations presented in the literature [28]. This fact can be important for the ruminant's health and milk composition, as alpha-tocopherol possesses the highest biological activity compared to gamma and delta-tocopherol isomers [29].

Table 3. The vitamin E isomers and total Vitamin E content of the white grape pomace

Parameters	WGP ¹
Alpha-tocopherol	69.87±0.066
Gamma-tocopherol	7.34±0.011
Delta-tocopherol	4.77±0.056
Total vitamin E	81.98±0.111

¹ White grape pomace

The remarkable composition of vitamin E in white grape pomace is noteworthy, given that the synthetic antioxidants are commonly used in ruminant diets to positively influence the storage time of milk and meat. However, concerns persist regarding the potential health effects of synthetic

compounds on consumers. As a result, there is a growing interest in exploring natural antioxidant sources as alternatives in ruminant nutrition [30]. The content of the water-soluble antioxidant compounds (total polyphenols and total flavonoids) is presented in Figure 2.

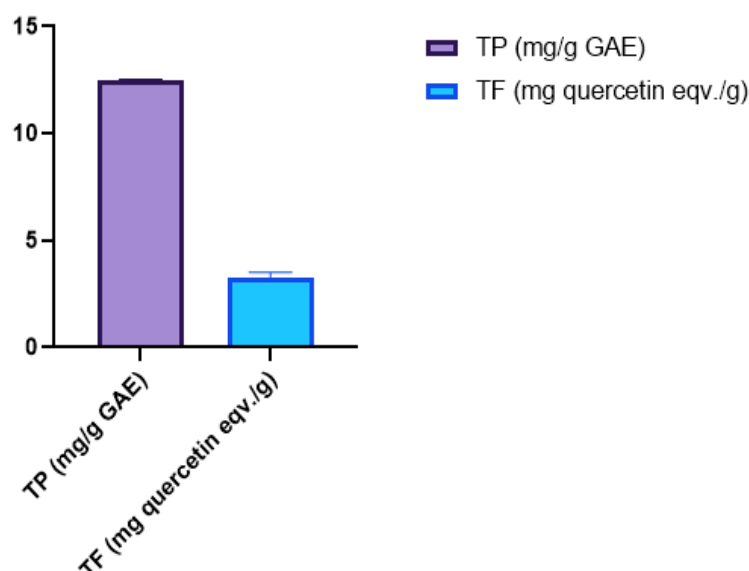


Figure 2. Total polyphenol and total flavonoid concentration of the white grape pomace

White grape pomace has been found to have a high concentration of total polyphenols, with low composition of total flavonoids, as shown in

Figure 2. The results of the total polyphenols content are consistent with the data presented in existing literature [23], whereas the content of

total flavonoids is comparatively lower. A possible explanation could be provided by the plant growth stage; studies showed that flavonoids exhibit a rich concentration in the skin and seeds of white grapes when they reach maturity. However, during the late maturity period, the concentration of flavonoids decreased. Additionally, the same trend has been observed for the white grape pulp, which showed total flavonoid values below the detection limit for the grapes that have not reached maturity but increased as the plant matured [31].

The inclusion of a natural polyphenol source in ruminants' nutrition can be beneficial, as the literature showed that they can influence the

inflammatory and oxidative state of the ruminants, which, at a higher level, can negatively impact the animals' performances and reproduction [32, 33]. In addition, *in vitro* experiments demonstrated that polyphenols exert a significant effect on reducing methane emissions, which might be linked to both energy losses (which lowers the animals' ability to store and utilize energy) and environmental issues [34].

It was necessary to investigate the antioxidant potential of white grape pomace due to its significant composition of antioxidant compounds. The results obtained from four different methods used to express the antioxidant capacity of it are presented in Figure 3.

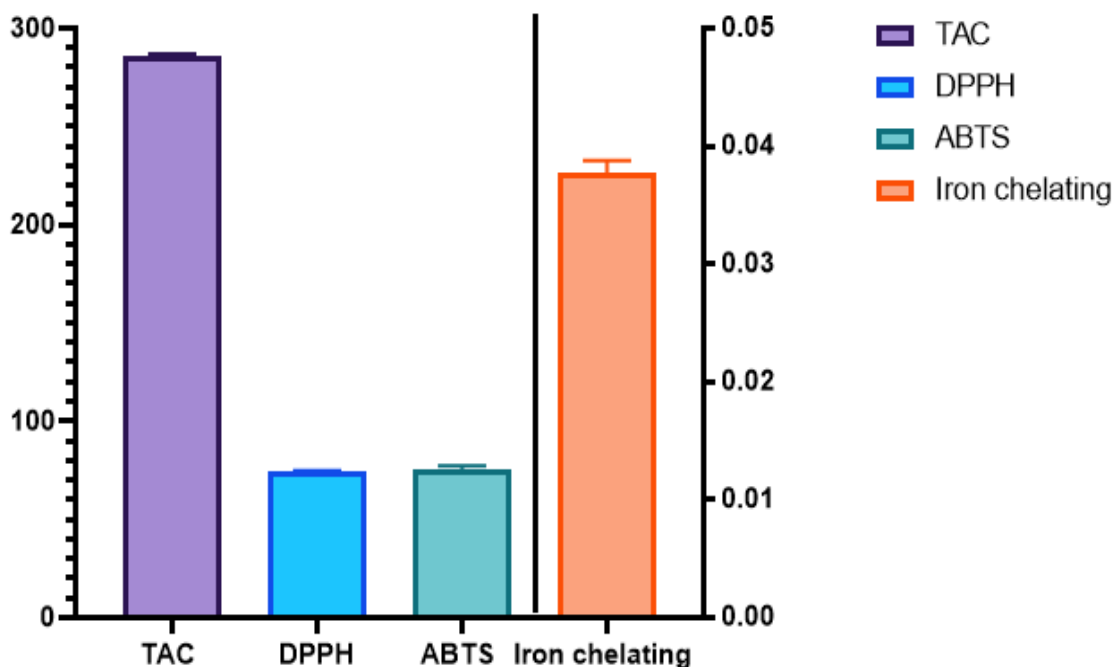


Figure 3. The antioxidant capacity of the white grape pomace, studied through four different methods; TAC (Ascorbic acid eqv.), DPPH (mmol Trolox eqv. /kg), ABTS (mmol Trolox eqv. /kg), Iron chelating (mg EDTA eqv. /g).

The total antioxidant capacity of white grape pomace was higher, consistent with the literature [23]. Additionally, it displayed impressive antioxidant potential as determined by DPPH and ABTS assays. In literature, there is considerable variability in the antioxidant potential of white grape pomace, which may be due to variations between grape cultivars and species [23].

Figure 4 presented the Pearson correlation as a correlation heatmap, between the fatty acids' composition (SFA, PUFA, MUFA, omega 6 and

omega 3 fatty acids), antioxidant compounds (total polyphenols, total flavonoids, and isomers of vitamin E), and antioxidant potential (total antioxidant capacity, DPPH, ABTS, and iron chelating capacity) for the white grape pomace. According to the heat map, the composition of the PUFA was strongly positively correlated with the composition of omega 3 and omega 6 fatty acids, $r=1$.

On the other hand, PUFA content was strongly negatively correlated with the composition of

delta-tocopherol, alpha-tocopherol, and total content of vitamin E ($r=-1$). Also, the antioxidant capacity of the white grape pomace, assayed

through TAC, DPPH, and iron chelating capacity, was strongly negatively correlated with the PUFA composition ($r=-1$).

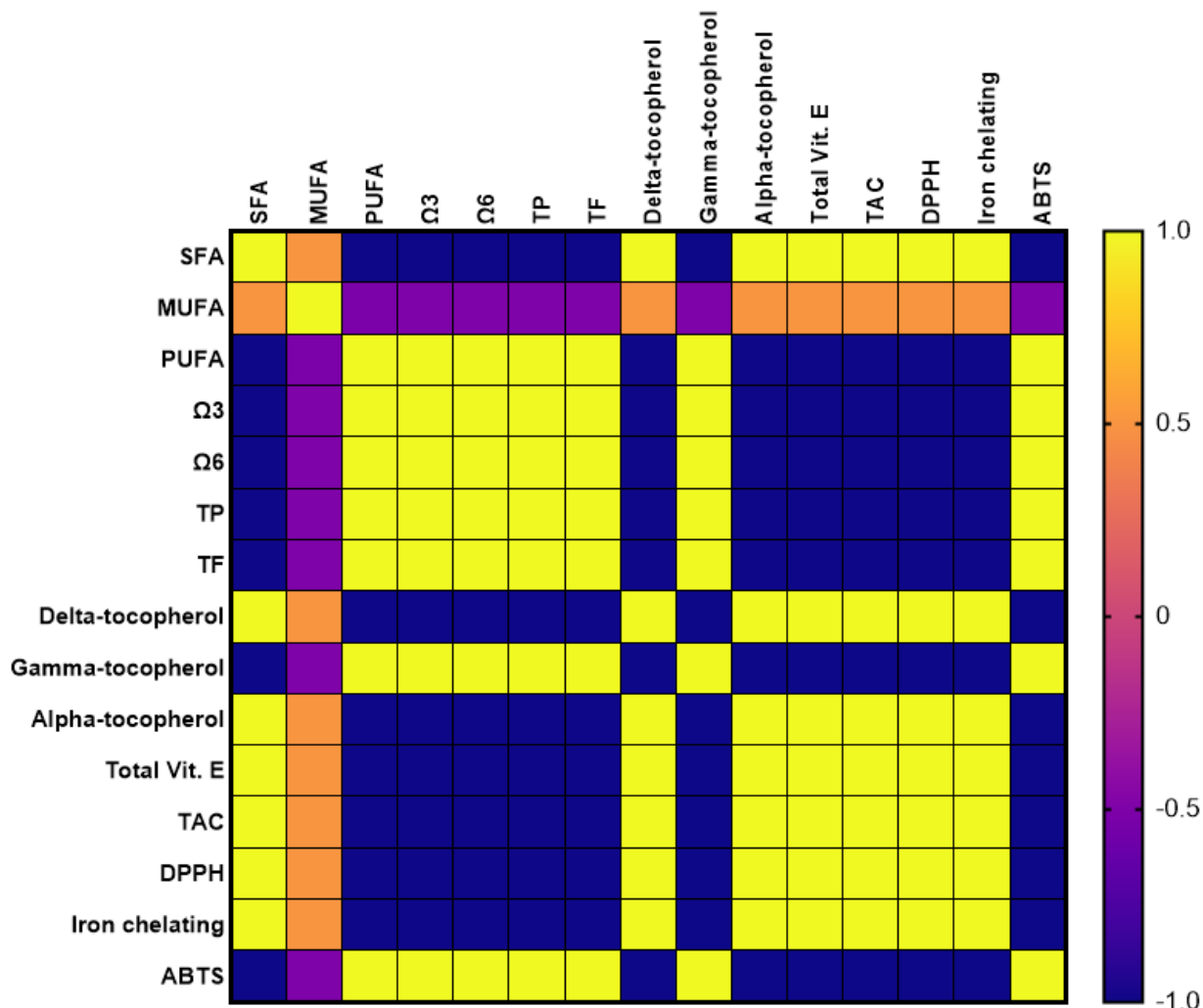


Figure 4. Pearson correlation as heatmap between the fatty acids' composition (SFA, PUFA, MUFA, omega 6 and omega 3 fatty acids), antioxidant compounds (total polyphenols, total flavonoids, and isomers of vitamin E), and antioxidant potential (total antioxidant capacity, DPPH, ABTS, and iron chelating capacity) for the white grape pomace.

The Pearson correlation also highlighted the strongly positive correlation between the antioxidant compounds and the analyses describing the antioxidant potential of the white grape pomace. Total polyphenol and total flavonoid content were positively correlated with ABTS assay ($r=1$), while the content of the alpha-, delta- tocopherol and total content of the vitamin E was positively correlated with TAC, DPPH, and iron chelating assays. Also, the content of the gamma-tocopherol was positively correlated with the ABTS assay. The results of the correlation

heat map showed a strongly positive correlation among TAC, DPPH, and iron chelating ability. However, this contrasts with previous literature which indicated a positive correlation between TAC, DPPH, and ABTS assays. Furthermore, the absence of a linear correlation between DPPH and ABTS, in line with both the literature and our observations, highlights the complexity of antioxidant interactions and highlights the necessity for further investigation into their mechanisms [23].

4. Conclusions

Our study revealed the nutritional richness of white grape pomace, highlighting its significant composition of essential fatty acids, particularly PUFA, such as linoleic and omega 6 fatty acids, along with a noteworthy presence of MUFA, predominantly oleic acid. Moreover, the analysis showed a remarkable concentration of total polyphenols and vitamin E, particularly in the form of alpha-tocopherol isomer, revealing its potential as a valuable source of antioxidants and essential nutrients. Through various assays including TAC, DPPH, and ABTS, it was revealed the high antioxidant potential of the studied white grape pomace. The important nutritional composition of white grape pomace, highlighted in this study, offers promising potential for its utilization in ruminant nutrition as a source of antioxidants and essential nutrients.

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