Original Paper

Fatty acid profile analysis of grape by-products from Slovakia and Austria

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The objective of the present study was to determine the fatty acid profile of grape pomace, grape stem and grape bunch of three different cultivars of *Vitis vinifera* sp. (Green Veltliner, Pinot Blanc and Zweigelt) from two countries as a possible sources for animal nutrition. Fatty acid profile analysis was performed using the Agilent 6890 A GC machine. Significant differences (P <0.05) in fatty acid content of analyzed samples were detected between the countries, as well as between the cultivars within countries. Grape pomaces and grape bunches were rich in polyunsaturated fatty acids (70.91–71.86%), represented mainly by linoleic acid (69.79–70.32%), and low in saturated fatty acids (12.42–12.96%). Grape stems were characterized by a high saturated fatty acids content (24.46–30.85%), but on the other hand, these samples had the highest α-linolec acid concentration (9.98–14.52%). Oleic acid (12.24–15.17%) was the most abundant from monounsaturated fatty acids (12.69–15.33%) in all the analyzed samples. These results indicate a strong impact of the grape variety and location on the fatty acid profile of grape by-products and their potential to be evaluated as feed additives with high polyunsaturated fatty acids concentration in animal nutrition.

Keywords: grape pomace, grape stalk, fatty acids, PUFA, SFA

1 Introduction

Grape industry generates a large amount of by-products with problematic disposal which can cause serious environmental issues (Botella et al., 2005, Rondeau et al., 2013, Bekhit et al., 2016). The two most abundant by-products of grape processing are pomace and stalks (Makris et al., 2007). Grape pomace represents about 20–25% of the weight of wine grapes (Yu and Ahmedna, 2013), the amount of stems can vary between 1.4–7% (Souquet et al., 2000). The nutritional value and the digestibility of these by-products is, due to high fiber content, generally low, but many experiments showed, that these products can be used a substantial source of certain nutrients and biologically active compounds in animal nutrition (Viveros et al., 2011, Teixeira et al., 2014, Chamorro et al., 2015, Domínguez et al., 2016, Kerasioti el al., 2017). They can also help to reduce production costs and to create innovative feed mixtures in order to increase the quality of animal products (Tangolar et al., 2009, Fontana et al., 2013, Guerra-Rivas et al., 2016, Kafantaris et al., 2018). According to Botella et al. (2005) the incorporation of winery by-products in livestock feeds may also positively affect the environment by reducing the toxic impact of their inappropriate disposal by leaving on open spaces or burning. Fatty acids of grape by-products, particularly those of grape pomace, are characterized with high concentrations of linoleic and oleic acids (Yi et al., 2009). Due to this fact, by-products of wine industry could positively influence the fatty acid profile of milk and meat, with a perspective of obtaining less saturated and healthier animal products (Nistor et al., 2014, Guerra-Rivas et al., 2016, Chedea et al., 2018). On this regard the objective

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of this study was to determine and compare the fatty acid profile of grape pomace, grape stems and grape bunches from two countries as possible sources of these nutrients for animal nutrition.

2 Material and methods

The pomace, as a by-product of juice pressing in wine industry, mainly contained of residual grape skin, seeds and pulps. Grape stems were only rachis, peduncle and pedicels after removing grape berries. In total, 54 samples from 3 varieties from 6 different locations were analysed. Laboratory samples were processed in the Laboratory of Quality and Nutritive Value of Feeds (Department of Animal Nutrition, Slovak University of Agriculture in Nitra) using standard laboratory procedures and principles (EC No 152/2009). Prior to evaluating the fatty acid profile of analyzed samples, triglycerides in their lipid fraction to glycerol and free fatty acids were hydrolyzed. Free fatty acids were then converted to methylesters (FAMEs) according to the following procedure. Solution was diluted by hexane (10 ml) and 2 N potassium hydroxide in methanol (1 ml). Analytic tube was heated in water bath (30 seconds at 60 °C). After 1 minute 1 N hydrochloric acid (2 ml) was added. The top layer was transmitted (2 ml) to autosampler vial containing ninhydrin (Na_2SO_4). On a specialized analytical column (Supelco 47885-U) the separation of FAMEs, based on the carbon number and level of saturation, took place. FAMEs with the shortest carbon chain (the lowest boiling point) were separated first. Subsequently, the individual fatty acids were identified by a flame ionization detector (FID). Analyis were performed on gas chromatograph Agilent 6890A GC (Agilent Technologies, USA). The fatty acids profile of grape by-products was determined as percentage of crude fat. Results were statistically evaluated with IBM SPSS v. 20.0. Descriptive statistics using one-way ANOVA were generated. Then, statistical significance of results were separated using Tukey test.

3 Results and discussion

The analyzed grape by-products were characterized by their specific fatty acid (FA) profiles (Table 1 and Table 2). Despite the significant (P <0.05) differences between the countries, as well as between the cultivars within countries, some similarities in the fatty acid composition of grape pomace, stems and bunches were detected. The samples mainly composed of polyunsaturated fatty acids (PUFA), mostly represented by linoleic acid, especially in grape pomace and grape bunches. This result is consistent with the grape seed content of these products as a source of linoleic acid rich grape oil (Fernandes et al., 2013, Yousefi et al., 2013, Hussein and Abdrabba, 2015, Ovcharova et al., 2016). In grape stems interesting content of α-linoleic acid was detected. Oleic acid, as a monounsaturated fatty acid (MUFA), was the most abundant in all the studied by-products. Grape stems contained the highest amount of saturated fatty acids (SFA), mainly palmitic and stearic acid. The high content of palmitic acid in pomaces may be due to surplus saturated compounds in their waxy structure (Gülcü et al., 2019). Arachidonic and behenic acid were present in pomaces below 1%, whereas in bunches these fatty acids, except two samples (Pinot Blanc and Zweigelt from Slovakia), were not found. This corresponds with low levels of SFA in grape seeds (Tangolar et al., 2009; Gül et al., 2013, Mironeasa et al., 2016, García-Lomillo and Gonzáles-San José, 2017).

The FA profile of grape pomace is well documented in the literature, but only a limited number of papers has been published on the content of FA in grape stem and grape bunch. In red grape pomace Yi et al. (2009) found average values of 21.2% SFA, 14.4% MUFA and 62.7% PUFA. Ribeiro et al. (2015) reported an average PUFA concentration in grape pomaces around 72.86% with the predominance of linoleic (60.04%) and α-linolenic (13.64%) acid, followed by oleic (12.97%) and palmitic (6.72%) acid. Stearic acid was present in the analyzed pomaces below 5%. In comparison with Guerra-Rivas et al. (2016) lower amounts of all the FA were detected for grape pomaces. On the other hand, Tsiplakou and Zervas (2008) and Gülcü et al. (2019) measured higher content of the same FA, except for linoleic acid. Russo et al. (2017) studied the FA profile of six grape pomaces with very similar results as obtained in this experiment. These authors also reported that grape stalk contained 21% palmitic, 4.6% stearic, 10.7% oleic, 35.4% linoleic, 13.4% α-linoleic and 11.3% behenic acid.

The total comparison of FA profile of grape by-products from Slovakia and Austria is shown in Table 3. The grape pomace samples from both countries had significantly different (P <0.05) content of all the studied FA. In the case of grape stems significant differences (P <0.05) for oleic, α-linoleic, arachidic and behenic acids concentration, as well as overall MUFA content, were found. The grape bunches from two counties significantly differed (P <0.05) in stearic, oleic, linoleic and α-linoleic acids content. A justification for this differences between the FA content of grape-by products could be related to different agro-climatic conditions of the growing regions (García-Lomillo and Gonzáles-San José, 2017). Bennemann et al. (2016) state, that the quality of grapes is greatly influenced by factors such as soil, weather, temperature, humidity and solar radiation.

Table 1 Fatty acid profile of grape by-products from Slovakia (% fat^{-1})

		Green Veltliner	Pinot Blanc	Zweigelt
		Mean ±Standard Deviation		
Palmitic acid	pomace	8.64 ±0.11[a]	8.13 ±0.01[b]	7.69 ±0.02[c]
	stems	15.80 ±0.45[a]	10.68 ±0.49[b]	13.14 ±0.68[c]
	bunch	8.85 ±0.02[a]	8.57 ±0.10[b]	7.47 ±0.05[c]
Stearic acid	pomace	3.56 ±0.05[a]	3.95 ±0.00[b]	4.03 ±0.00[c]
	stems	3.52 ±0.21[a]	4.03 ±0.18[b]	3.86 ±0.05[ab]
	bunch	3.42 ±0.02[a]	4.06 ±0.03[b]	4.17 ±0.01[c]
Oleic acid	pomace	10.91 ±0.07[a]	17.52 ±0.02[b]	16.34 ±0.02[c]
	stems	14.34 ±0.92[a]	16.12 ±0.12[b]	15.04 ±0.31[ab]
	bunch	10.21 ±0.01[a]	17.09 ±0.15[b]	17.03 ±0.06[c]
Linoleic acid	pomace	73.08 ±0.23[a]	67.59 ±0.02[b]	68.75 ±0.01[c]
	stems	36.86 ±1.14[a]	57.19 ±1.21[b]	45.47 ±1.05[c]
	bunch	74.40 ±0.03[a]	67.66 ±0.25[b]	68.90 ±0.04[c]
α-linoleic acid	pomace	1.75 ±0.02[a]	0.78 ±0.00[b]	0.77 ±0.01[b]
	stems	15.17 ±0.62[a]	5.74 ±0.13[b]	9.03 ±0.62[c]
	bunch	2.38 ±0.03[a]	1.22 ±0.06[b]	1.01 ±0.05[c]
Arachidic acid	pomace	0.28 ±0.01[a]	0.24 ±0.00[b]	0.24 ±0.00[b]
	stems	3.21 ±0.07[a]	1.21 ±0.03[b]	2.89 ±0.09[c]
	bunch	ND[a]	0.24 ±0.00[b]	0.25 ±0.00[c]
Behenic acid	pomace	0.19 ±0.01[a]	0.11 ±0.00[b]	0.11 ±0.00[b]
	stems	3.62 ±0.11[a]	1.95 ±0.08[b]	4.76 ±0.15[c]
	bunch	ND	ND	ND
PUFA	pomace	74.83 ±0.25[a]	68.37 ±0.02[b]	69.52 ±0.02[c]
	stems	54.26 ±1.81[a]	62.93 ±1.15[b]	54.51 ±0.46[a]
	bunch	76.78 ±0.03[a]	68.88 ±0.18[b]	69.91 ±0.08[c]
MUFA	pomace	11.32 ±0.08[a]	17.95 ±0.02[b]	16.72 ±0.02[c]
	stems	14.34 ±0.92[a]	16.31 ±0.43[b]	15.04 ±0.31[ab]
	bunch	10.21 ±0.01[a]	17.39 ±0.15[b]	17.34 ±0.06[b]
SFA	pomace	12.93 ±0.18[a]	12.57 ±0.02[b]	12.30 ±0.01[c]
	stems	28.87 ±0.78[a]	18.45 ±0.73[b]	26.06 ±0.71[c]
	bunch	12.28 ±0.02[a]	12.98 ±0.29[b]	11.99 ±0.15[a]
Ratio Σn3/n6	pomace	0.02 ±0.00[a]	0.01 ±0.00[b]	0.01 ±0.00[b]
	stems	0.43 ±0.00[a]	0.10 ±0.00[b]	0.20 ±0.02[c]
	bunch	0.03 ±0.00[a]	0.02 ±0.00[b]	0.01 ±0.00[b]
Ratio Σn3/n6	pomace	41.72 ±0.47[a]	86.60 ±0.13[b]	89.59 ±0.75[c]
	stems	2.34 ±0.03[a]	9.96 ±0.39[b]	5.05 ±0.47[c]
	bunch	31.31 ±0.37[a]	55.34 ±2.88[b]	68.26 ±3.48[c]

ND – value below detection limit, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids. Values followed by different letters within a row are significant at the level 0.05

Table 2 Fatty acid profile of grape by-products from Austria (% fat^{-1})

		Green Veltliner	Pinot Blanc	Zweigelt
		Mean ±Standard Deviation		
Palmitic acid	pomace	8.85 ±0.01[a]	7.96 ±0.03[b]	8.70 ±0.03[c]
	stems	19.13 ±0.15	16.79 ±0.31	19.62 ±5.85
	bunch	9.89 ±0.28	7.69 ±0.03	9.86 ±1.56
Stearic acid	pomace	3.25 ±0.01[a]	3.44 ±0.01[b]	3.77 ±0.01[c]
	stems	3.92 ±0.11	4.35 ±0.11	5.75 ±2.05
	bunch	3.60 ±0.07[a]	3.36 ±0.02[a]	4.42 ±0.51[b]
Oleic acid	pomace	9.80 ±0.04[a]	15.98 ±0.03[b]	15.86 ±0.01[c]
	stems	9.43 ±0.74[a]	12.04 ±0.23[ab]	15.26 ±2.69[b]
	bunch	10.96 ±0.10[a]	16.39 ±0.05[b]	16.86 ±0.51[b]
Linoleic acid	pomace	73.85 ±0.09[a]	68.89 ±0.10[b]	66.61 ±0.04[c]
	stems	38.92 ±1.07	36.06 ±0.29	38.40 ±6.70
	bunch	72.98 ±0.39[a]	70.63 ±0.11[a]	66.82 ±2.41[b]
α-linoleic acid	pomace	1.81 ±0.01[a]	1.15 ±0.03[b]	1.21 ±0.02[c]
	stems	18.73 ±1.05[a]	15.65 ±0.21[a]	9.17 ±2.47[b]
	bunch	1.84 ±0.02[a]	1.09 ±0.07[b]	1.13 ±0.08[b]
Arachidic acid	pomace	0.23 ±0.01[a]	0.34 ±0.01[b]	0.27 ±0.01[c]
	stems	2.41 ±0.09[a]	3.52 ±0.07[b]	2.30 ±0.24[a]
	bunch	ND	ND	ND
Behenic acid	pomace	0.17 ±0.00[a]	0.24 ±0.00[b]	0.17 ±0.00[a]
	stems	3.81 ±0.20[a]	5.73 ±0.16[b]	3.71 ±0.48[a]
	bunch	ND	ND	ND
PUFA	pomace	75.66 ±0.10[a]	70.04 ±0.10[b]	67.82 ±0.04[c]
	stems	57.66 ±0.39	51.71 ±0.42	47.57 ±9.10
	bunch	74.83 ±0.39[a]	71.72 ±0.09[a]	67.95 ±2.48[b]
MUFA	pomace	10.09 ±0.05[a]	16.50 ±0.03[b]	16.60 ±0.01[c]
	stems	9.43 ±0.74[a]	12.04 ±0.23[a]	16.61 ±2.48[b]
	bunch	10.96 ±0.10[a]	16.39 ±0.05[b]	17.00 ±0.39[c]
SFA	pomace	12.89 ±0.01[a]	12.38 ±0.04[b]	13.37 ±0.04[c]
	stems	29.27 ±0.34	30.39 ±0.29	32.88 ±7.35
	bunch	13.49 ±0.31[ab]	11.06 ±0.04[a]	14.28 ±2.07[b]
Ratio Σn3/n6	pomace	0.02 ±0.00[a]	0.02 ±0.00[b]	0.02 ±0.00[c]
	stems	0.48 ±0.04[a]	0.43 ±0.01[a]	0.24 ±0.03[b]
	bunch	0.03 ±0.00[a]	0.02 ±0.00[b]	0.02 ±0.00[b]
Ratio Σn3/n6	pomace	40.778 ±0.27[a]	60.16 ±1.34[b]	55.25 ±0.77[c]
	stems	2.08 ±0.18[a]	2.30 ±0.03[a]	4.28 ±0.56[b]
	bunch	39.56 ±0.59[a]	65.07 ±4.09[b]	59.20 ±2.07[b]

ND – value below detection limit, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids. Values followed by different letters within a row are significant at the level 0.05

Table 3 Comparison of fatty acid profile of grape by-products from Slovakia and Austria

		Slovakia	Austria	Significance
		Mean ±Standard deviation (% fat^{-1})		
Palmitic acid	pomace	8.15 ±0.41	8.50 ±0.41	0.000
	stems	13.21 ±2.27	18.51 ±3.21	0.578
	bunch	8.30 ±0.64	9.15 ±1.35	0.041
Stearic acid	pomace	3.84 ±0.22	3.49 ±0.23	0.000
	stems	3.80 ±0.26	4.67 ±1.32	0.223
	bunch	3.88 ±0.35	3.79 ±0.54	0.012
Oleic acid	pomace	14.92 ±3.06	13.88 ±3.06	0.000
	stems	15.17 ±0.92	12.24 ±2.89	0.013
	bunch	14.77 ±3.43	14.74 ±2.85	0.000
Linoleic acid	pomace	69.81 ±2.51	69.79 ±3.21	0.000
	stems	46.51 ±8.89	37.80 ±3.64	0.656
	bunch	70.32 ±3.11	70.14 ±2.96	0.005
α-linoleic acid	pomace	1.10 ±0.49	1.39 ±0.32	0.000
	stems	9.98 ±4.17	14.52 ±4.44	0.001
	bunch	1.54 ±0.64	1.35 ±0.37	0.000
Arachidic acid	pomace	0.25 ±0.02	0.28 ±0.05	0.000
	stems	2.44 ±0.93	2.74 ±0.60	0.000
	bunch	0.16 ±0.12	ND	ND
Behenic acid	pomace	0.14 ±0.04	0.20 ±0.03	0.000
	stems	3.44 ±1.23	4.41 ±1.02	0.000
	bunch	ND	ND	ND
PUFA	pomace	70.91 ±2.99	71.17 ±3.50	0.000
	stems	57.23 ±4.41	52.31 ±6.33	0.140
	bunch	71.86 ±3.72	71.50 ±3.24	0.003
MUFA	pomace	15.33 ±3.06	14.40 ±3.23	0.000
	stems	15.23 ±1.01	12.69 ±3.40	0.003
	bunch	14.98 ±3.58	14.78 ±2.89	0.000
SFA	pomace	12.60 ±0.29	12.88 ±0.43	0.000
	stems	24.46 ±4.71	30.85 ±4.02	0.594
	bunch	12.42 ±0.47	12.94 ±1.79	0.039
Ratio Σn3/n6	pomace	0.02 ±0.01	0.02 ±0.00	0.000
	stems	0.24 ±0.15	0.38 ±0.12	0.000
	bunch	0.02 ±0.01	0.19 ±0.00	0.000
Ratio Σn3/n6	pomace	72.64 ±23.23	52.06 ±8.76	0.000
	stems	5.78 ±3.36	2.89 ±1.09	0.000
	bunch	51.64 ±16.40	54.61 ±11.80	0.000

ND – value below detection limit, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids. The level of significance was set at $P < 0.05$

4 Conclusions

The results of this experimnet indicate a significant impact of the grape variety and location on the FA profile of grape by-products. But despite these differences some similarities can be found. Grape pomaces and grape bunches were rich in PUFA, especially linoleic acid, and low in SFA. Grape stems were characterized by a high SFA content, but on the other hand, these samples had the highest *H*-linolec acid concentration. Overall it can ce concluded that the by-products of wine industry, primarily grape pomace, could find application in animal nutrition as feed additives with high PUFA content. However, further research in the future in needed.

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