

### THE AMINO ACIDS CONTENT IN GRAPE BY-PRODUCTS FROM SLOVAKIA AND AUSTRIA

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#### ABSTRACT

The aim of the experiment was to determine the amino acid content in 3 grape varieties by-products (bunch, pomace, stem) from Austria (AT) and Slovakia (SK). In total, 54 samples from 3 varieties Zweigelt (ZG), Pinot Blanc (PB) and Green Veltliner (GV) white skinned from 6 different locations (Nitra and Vienna wine region) of *Vitis vinifera* sp. were analysed. In each variety the amino acids composition was determined for the whole grape (bunch) and by-products of wine industry (pomace, stems). Samples of grape by-products were freeze dried, homogenized and analysed for amino acids concentration using amino acid analyzer AAA 400 (Ingos, Prague). Descriptive statistics (means, standard error of mean, minimum maximum), differences between varieties (Tukey test,  $p < 0.05$ ) using One-way ANOVA and differences between the countries with independent samples T-test ( $p < 0.05$ ) by IBM SPSS v. 20.0 were generated. After the amino acid analysis in grape bunch, pomace and stem in general the highest content of glutamic and aspartic acid was found. However, the grape pomace amino acid content is highly variable and statistically different between the varieties and countries. In contrary, lower variability in amino acids content between the varieties was found in grape stem. However, similar pattern in amino acids with the lowest content between the countries and varieties in grape by-products was observed. Finally, low content of Cysteine, Methionine, Histidine and Proline was found.

**Keywords:** amino acids, grape bunch, grape pomace, grape stem

#### INTRODUCTION

Wine producing process is known to generate large amounts of residual parts (Brahim *et al.*, 2014) like grape pomace or grape marc and grape stems or stalks (Barcia *et al.*, 2014). These by-products, which could be used in animal nutrition as a beneficial additive because of the bioactive compounds content (Poveda *et al.*, 2018). Because of higher content of water in fresh state is necessary to conserve grape by-products for preservation of nutrients. For example, Juráček *et al.* (2019) conserved grape pomace by silaging process with possibility to use urea as conservation additive. Then, Oprica *et al.* (2019) mentioned as a good method for grape bioactive compounds preservation oven-drying and freeze-drying. Grape pomace is source of crude proteins, ether extracts and crude fibre. Grape stem has high concentration of hemicellulose, cellulose, total phenols, condensed tannins and the highest antioxidant activity from grape by-products (Hanusovský *et al.*, 2020). However, the nutritional composition of grape samples shown that it varies widely, depending on the grape variety, grape origin and the conditions of fertilization (Brenes *et al.*, 2016). Also, the nutritive value of grape by-products is determined by ratio of seeds and pulps (Guerrera-Rivas *et al.*, 2016). Differences are mainly in the content of crude proteins, ether extracts and fibre complex (Hanusovský *et al.*, 2019). Then, grape by-products are good source of minerals - for example potassium, calcium and iron but the locality can affect mainly the content of calcium, sodium, potassium, zinc and iron (Šimko *et al.*, 2019). After that, Kolláthová *et al.* (2020) found that grape pomaces and grape bunches were rich in polyunsaturated fatty acids (PUFA), especially linoleic acid, and low in saturated fatty acids (SFA). Grape stems were characterized by a high SFA content, but on the other hand, these samples had the highest H-linoleic acid concentration. Another bioactive substances presented in red, white and grape by-products are hydroxycinnamic tartaric acids, hydroxybenzoic acids, flavanols, flavonols, anthocyanins and stilbenoids which are main classes of phenols (Giuffrè, 2013; Rešičič *et al.*, 2016). However, because experiments focused on the amino acid content in grape by-products are outdated or missing, the aim of the experiment was to determine the amino acid content in 3 grape varieties by-products (bunch, pomace, stem) from Austria and Slovakia.

#### MATERIAL AND METHODS

##### Grape samples

In total, 54 samples from 3 varieties Zweigelt (ZG) red skinned, Pinot Blanc (PB) white skinned and Green Veltliner (GV) white skinned from 6 different locations (Nitra and Vienna wine region) of *Vitis vinifera* sp. were analysed. The microclimatic conditions in Nitra wine region (average temperature 10.8 °C, precipitations 988.06 mm and 95 days in rain) and Vienna wine region (average temperature 12.0 °C, precipitations 716.28 mm and 74 days in rain) by rp5.ru meteorological server were observed. In each variety the amino acids composition was determined for the whole grape (bunch) and by-products of wine industry (pomace, stems). Grape pomace was characterised as a residual skin, seeds and grape pulps after juice pressing in wine industry. Grape stems were only rachis, peduncle and pedicels after removing grape berries.

##### Chemical analysis

Samples of grape by-products were freeze dried, homogenized, and analysed for amino acids concentration using amino acid analyzer AAA 400 (Ingos, Prague). Then, in first step samples for determination of amino acids by the acidic and oxidative hydrolysis were hydrolysed. Then were analysed by HPLC with post column ninhydrin derivatisation (AAA 400).

##### Statistical analysis of results

Results were statistically evaluated with IBM SPSS v. 20.0. Descriptive statistics (means, standard error of mean, minimum maximum) using One-way ANOVA were generated. Then, statistical significance of amino acids between the varieties (3 samples per variety in both countries) within the countries was expressed using Tukey test ( $p < 0.05$ ). The differences between the countries were evaluated by independent samples T-test ( $p < 0.05$ ).

**RESULTS**

As is shown in the Table 1, significant differences mainly between the varieties in amino acid content were found. However, higher variability and differences in Austrian (AT) samples in comparison with Slovakian (SK) samples were observed. For example, the content of Aspartic acid, Glycine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Lysine and Cysteine was significantly different and variable between all varieties. On the other hand, in all SK samples was significantly different only content of Aspartic acid and Arginine. After the comparison of the amino acid content between the countries statistically significant differences between the varieties except of Aspartic acid in GV and ZG, Alanine in ZG, Phenylalanine in GV, Arginine in GV, Cysteine in PB and Methionine in PB and

ZG variety were observed. In GV and PB variety were the most represented amino acids Aspartic and Glutamic acids in AT samples. In contrary, in AT samples, Proline was presented only in ZG variety but in highest quantity in comparison with other amino acids. Similarly like in AT GV and PB samples, the highest content from amino acids in SK grape bunch samples was represented by Aspartic and Glutamic acid. However, similar pattern in amino acids with the lowest content between the countries and varieties was observed. Thus, the content of Proline, Methionine and Cysteine in GV, PB varieties in AT samples and in GV, ZG varieties in SK samples was the lowest - even in the same order. Exceptions were ZG variety in AT samples with the lowest content of Isoleucine and PB variety from SK where was limiting amino acid Methionine.

**Table 1** Differences in the amino acid content between the varieties and countries in whole grape bunch in g.kg<sup>-1</sup> dry matter

AA	AT						SK					
	Mean			SE	Min	Max	Mean			SE	Min	Max
	GV	PB	ZG				GV	PB	ZG			
asp	4.06 <sup>a*</sup>	3.22 <sup>b</sup>	2.69 <sup>a*</sup>	0.21	2.56	4.26	3.99 <sup>a*</sup>	10.76 <sup>b</sup>	2.55 <sup>c*</sup>	1.27	2.38	11.19
thr	1.28 <sup>a</sup>	0.87 <sup>b</sup>	0.85 <sup>b</sup>	0.07	0.80	1.34	1.01 <sup>a</sup>	3.99 <sup>b</sup>	1.00 <sup>a</sup>	0.50	0.93	4.15
ser	1.51 <sup>a</sup>	1.06 <sup>b</sup>	0.97 <sup>b</sup>	0.08	0.92	1.58	1.22 <sup>a</sup>	4.88 <sup>b</sup>	1.22 <sup>a</sup>	0.61	1.14	5.07
glu	4.05 <sup>a</sup>	4.16 <sup>a</sup>	2.72 <sup>b</sup>	0.24	2.59	4.45	3.57 <sup>a</sup>	14.08 <sup>b</sup>	3.35 <sup>a</sup>	1.77	3.11	14.64
pro	0.00 <sup>a</sup>	0.00 <sup>a</sup>	5.57 <sup>b</sup>	0.93	0.00	5.85	0.00 <sup>a</sup>	4.95 <sup>b</sup>	0.00 <sup>a</sup>	0.83	0.00	5.15
gly	1.80 <sup>a</sup>	1.22 <sup>b</sup>	0.99 <sup>c</sup>	0.12	0.94	1.89	1.39 <sup>a</sup>	5.77 <sup>b</sup>	1.44 <sup>a</sup>	0.73	1.31	6.00
ala	1.47	1.66	1.45 <sup>*</sup>	0.04	1.38	1.78	1.28 <sup>a</sup>	4.62 <sup>b</sup>	1.44 <sup>a*</sup>	0.54	1.21	4.80
val	1.14 <sup>a</sup>	0.83 <sup>b</sup>	0.82 <sup>b</sup>	0.05	0.78	1.20	0.97 <sup>a</sup>	4.15 <sup>b</sup>	1.02 <sup>a</sup>	0.53	0.91	4.32
ile	0.98 <sup>a</sup>	0.76 <sup>b</sup>	0.17 <sup>c</sup>	0.12	0.16	1.03	0.76 <sup>a</sup>	3.13 <sup>b</sup>	0.83 <sup>a</sup>	0.39	0.72	3.25
leu	1.97 <sup>a</sup>	1.53 <sup>b</sup>	0.68 <sup>c</sup>	0.19	0.65	2.07	1.62 <sup>a</sup>	6.49 <sup>b</sup>	1.73 <sup>a</sup>	0.80	1.52	6.75
tyr	1.23 <sup>a</sup>	0.74 <sup>b</sup>	1.45 <sup>c</sup>	0.11	0.69	1.52	1.06 <sup>a</sup>	2.88 <sup>b</sup>	1.03 <sup>a</sup>	0.31	0.96	3.00
phe	2.04 <sup>a*</sup>	1.79 <sup>b</sup>	1.01 <sup>c</sup>	0.16	0.96	2.14	1.81 <sup>a*</sup>	4.47 <sup>b</sup>	1.74 <sup>a</sup>	0.45	1.61	4.65
his	1.09 <sup>a</sup>	0.98 <sup>a</sup>	1.77 <sup>b</sup>	0.13	0.91	1.86	0.80 <sup>a</sup>	2.56 <sup>b</sup>	0.75 <sup>a</sup>	0.30	0.69	2.66
lys	1.47 <sup>a</sup>	0.92 <sup>b</sup>	0.56 <sup>c</sup>	0.13	0.53	1.54	1.11 <sup>a</sup>	5.80 <sup>b</sup>	1.17 <sup>a</sup>	0.78	1.04	6.03
arg	2.53 <sup>a*</sup>	2.56 <sup>a</sup>	0.98 <sup>b</sup>	0.26	0.93	2.74	2.64 <sup>a*</sup>	6.22 <sup>b</sup>	2.09 <sup>c</sup>	0.65	1.94	6.47
cysH	0.79 <sup>a</sup>	0.55 <sup>b*</sup>	0.44 <sup>c</sup>	0.05	0.42	0.83	0.68	0.61 <sup>*</sup>	0.60	0.02	0.56	0.72
metS	0.64 <sup>a</sup>	0.45 <sup>b*</sup>	0.44 <sup>b*</sup>	0.03	0.42	0.67	0.40 <sup>a</sup>	0.42 <sup>a*</sup>	0.50 <sup>b*</sup>	0.02	0.38	0.53

abbreviations: AA: aminoacid, AT: Austria, SK: Slovakia, GV: Green Veltliner, PB: Pinot Blanc, ZG: Zweigelt, SE: standard error of mean, Min: minimal value, Max: maximal value, asp: aspartic acid, thr: threonine, ser: serine, glu: glutamic acid, pro: proline, gly: glycine, ala: alanine, val: valine, ile: isoleucine, leu: leucine, tyr: tyrosine, phe: phenylalanine, his: histidine, lys: lysine, arg: arginine, cysH: cysteine, metS: methionine. The letters in superscripts indicates statistically significance of differences at the level 0.05 (Tukey test). Differences between countries within the varieties are marked by \* in superscripts at the level 0.05 (Independent samples T-test).

The grape pomace amino acid content is also highly variable and statistically different between the varieties and countries (Table 2). For example, statistically different results in the content of Serine, Glutamic acid, Glycine, Alanine, Valine, Isoleucine, Phenylalanine, Histidine and Methionine between the varieties and countries were found. Moreover, nonsignificant differences between the AT and SK samples only in Phenylalanine (PB variety), Arginine (GV variety) and Cysteine content (ZG variety) were observed. However, similar pattern in amino acids content between the varieties and countries was found. The most represented amino acid in AT and SK samples in GV and PB variety was Glutamic acid. In contrary, in ZG variety from AT the most represented amino acid was Proline and

from SK Arginine. Also, in both countries higher content of Aspartic acid was determined. On the other side, in ZG grape pomace variety from SK sample had higher content of Cysteine and Methionine. Similar pattern in both countries in the term of the lowest amino acid content was observed. Thus, in the grape pomace low content of Cysteine, Methionine, Histidine and Proline was found. The exception was ZG variety from SK with the lower concentration of Aspartic acid and Isoleucine but also with the lowest content of Proline like GV and PB sample from AT.

**Table 2** Differences in the amino acid content between the varieties and countries in grape pomace in g.kg<sup>-1</sup> dry matter

AA	AT					SK						
	Mean			SE	Min	Max	Mean			SE	Min	Max
	GV	PB	ZG				GV	PB	ZG			
asp	6.14 <sup>a</sup>	6.16 <sup>a</sup>	12.62 <sup>b</sup>	1.08	5.84	13.00	9.08 <sup>a</sup>	9.78 <sup>b</sup>	0.47 <sup>c</sup>	1.50	0.46	10.07
thr	2.35 <sup>a</sup>	2.67 <sup>b</sup>	5.24 <sup>c</sup>	0.46	2.23	5.40	3.25 <sup>a</sup>	3.20 <sup>a</sup>	1.64 <sup>b</sup>	0.26	1.59	3.31
ser	2.84 <sup>a</sup>	3.28 <sup>b</sup>	6.12 <sup>c</sup>	0.51	2.70	6.30	3.82 <sup>a</sup>	4.05 <sup>b</sup>	0.82 <sup>c</sup>	0.52	0.80	4.17
glu	7.71 <sup>a</sup>	9.49 <sup>b</sup>	14.15 <sup>c</sup>	0.97	7.32	14.58	9.86 <sup>a</sup>	13.58 <sup>b</sup>	1.49 <sup>c</sup>	1.79	1.45	13.98
pro	0.00 <sup>a</sup>	0.00 <sup>a</sup>	14.45 <sup>b</sup>	2.41	0.00	14.89	4.60 <sup>a</sup>	4.75 <sup>a</sup>	0.00 <sup>b</sup>	0.78	0.00	4.90
gly	3.44 <sup>a</sup>	4.22 <sup>b</sup>	6.11 <sup>c</sup>	0.40	3.27	6.29	4.41 <sup>a</sup>	5.52 <sup>b</sup>	1.46 <sup>c</sup>	0.61	1.42	5.68
ala	2.79 <sup>a</sup>	3.42 <sup>b</sup>	5.96 <sup>c</sup>	0.49	2.65	6.14	3.80 <sup>a</sup>	4.02 <sup>b</sup>	1.10 <sup>c</sup>	0.47	1.07	4.14
val	2.33 <sup>a</sup>	2.72 <sup>b</sup>	5.28 <sup>c</sup>	0.46	2.22	5.43	3.13 <sup>a</sup>	3.36 <sup>b</sup>	0.91 <sup>c</sup>	0.39	0.88	3.46
ile	1.91 <sup>a</sup>	2.27 <sup>b</sup>	4.13 <sup>c</sup>	0.35	1.81	4.26	2.45 <sup>a</sup>	2.59 <sup>b</sup>	0.75 <sup>c</sup>	0.30	0.73	2.67
leu	3.93 <sup>a</sup>	4.67 <sup>b</sup>	8.37 <sup>c</sup>	0.69	3.74	8.62	5.22 <sup>a</sup>	5.51 <sup>a</sup>	1.58 <sup>b</sup>	0.63	1.53	5.68
tyr	1.95 <sup>a</sup>	2.05 <sup>a</sup>	3.65 <sup>b</sup>	0.28	1.85	3.76	2.48 <sup>a</sup>	2.38 <sup>a</sup>	1.10 <sup>b</sup>	0.22	1.07	2.53
phe	3.49 <sup>a</sup>	4.13 <sup>b*</sup>	5.66 <sup>c</sup>	0.33	3.32	5.83	4.08 <sup>a</sup>	4.35 <sup>b*</sup>	1.68 <sup>c</sup>	0.42	1.63	4.48
his	1.98 <sup>a</sup>	2.31 <sup>b</sup>	3.37 <sup>c</sup>	0.21	1.88	3.47	2.29 <sup>a</sup>	2.63 <sup>b</sup>	0.77 <sup>c</sup>	0.29	0.74	2.71
lys	3.22 <sup>a</sup>	3.60 <sup>a</sup>	7.91 <sup>b</sup>	0.75	3.06	8.14	3.95 <sup>a</sup>	4.25 <sup>b</sup>	0.94 <sup>c</sup>	0.53	0.91	4.38
arg	6.16 <sup>a*</sup>	6.20 <sup>a</sup>	6.99 <sup>b</sup>	0.15	5.86	7.20	6.56 <sup>a*</sup>	7.63 <sup>b</sup>	2.32 <sup>c</sup>	0.81	2.25	7.85
cysH	1.34 <sup>a</sup>	1.43 <sup>a</sup>	2.23 <sup>b*</sup>	0.14	1.27	2.29	1.69 <sup>a</sup>	1.82 <sup>a</sup>	2.24 <sup>b*</sup>	0.08	1.66	2.31
metS	1.08 <sup>a</sup>	1.25 <sup>b</sup>	2.34 <sup>c</sup>	0.20	1.03	2.41	1.45 <sup>a</sup>	1.64 <sup>b</sup>	1.91 <sup>c</sup>	0.07	1.42	1.97

abbreviations: AA: aminoacid, AT: Austria, SK: Slovakia, GV: Green Veltliner, PB: Pinot Blanc, ZG: Zweigelt, SE: standard error of mean, Min: minimal value, Max: maximal value, asp: aspartic acid, thr: threonine, ser: serine, glu: glutamic acid, pro: proline, gly: glycine, ala: alanine, val: valine, ile: isoleucine, leu: leucine, tyr: tyrosine, phe: phenylalanine, his: histidine, lys: lysine, arg: arginine, cysH: cysteine, metS: methionine. The letters in superscripts indicates statistically significance of differences at the level 0.05 (Tukey test). Differences between countries within the varieties are marked by \* in superscripts at the level 0.05 (Independent samples T-test).

In comparison with whole grape bunch and pomace lower variability in amino acids content between the varieties was found (Table 3). Statistically significant differences between all varieties only in Methionine content in AT samples and Aspartic acid, Glutamic acid and Histidine in SK samples were determined.

However, between the countries nonsignificant differences only in Glutamic acid and Tyrosine content in ZG variety were observed. Then, in grape stem in both countries, the highest content of Aspartic acid, Glutamic acid and Arginine was found except of ZG variety from SK with higher content of Leucine instead of

Arginine. On the other side, the limiting amino acids were Cysteine, Methionine and Proline in both countries and in all varieties except of GV variety from SK. In GV grape stem the lowest content from amino acids was represented by Histidine.

**Table 3** Differences in the amino acid content between the varieties and countries in grape stem in g.kg<sup>-1</sup> dry matter

AA	AT						SK					
	Mean			SE	Min	Max	Mean			SE	Min	Max
	GV	PB	ZG				GV	PB	ZG			
asp	4.34 <sup>a</sup>	4.29 <sup>a</sup>	5.01 <sup>b</sup>	0.14	4.03	5.31	6.11 <sup>a</sup>	7.27 <sup>b</sup>	4.42 <sup>c</sup>	0.42	4.25	7.49
thr	1.45 <sup>a</sup>	1.65 <sup>ab</sup>	1.83 <sup>b</sup>	0.06	1.40	1.94	2.89 <sup>a</sup>	2.38 <sup>b</sup>	2.12 <sup>b</sup>	0.12	2.04	3.03
ser	1.65 <sup>a</sup>	1.86 <sup>ab</sup>	2.07 <sup>b</sup>	0.07	1.59	2.20	3.30 <sup>a</sup>	2.64 <sup>b</sup>	2.50 <sup>b</sup>	0.13	2.40	3.46
glu	3.55 <sup>a</sup>	4.96 <sup>b</sup>	4.80 <sup>bf</sup>	0.23	3.40	5.26	9.48 <sup>a</sup>	12.36 <sup>b</sup>	4.64 <sup>ac*</sup>	1.13	4.46	12.73
pro	0.00	0.00	0.00	0.00	0.00	0.00	3.98 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.66	0.00	4.18
gly	1.58 <sup>a</sup>	1.75 <sup>ab</sup>	1.93 <sup>b</sup>	0.06	1.51	2.05	2.94 <sup>a</sup>	2.54 <sup>b</sup>	2.32 <sup>b</sup>	0.10	2.23	3.09
ala	1.70 <sup>a</sup>	1.91 <sup>ab</sup>	2.09 <sup>b</sup>	0.06	1.64	2.21	3.17 <sup>a</sup>	2.79 <sup>b</sup>	2.52 <sup>b</sup>	0.10	2.42	3.33
val	1.33 <sup>a</sup>	1.43 <sup>a</sup>	1.70 <sup>b</sup>	0.06	1.28	1.80	2.62 <sup>a</sup>	2.10 <sup>b</sup>	2.02 <sup>b</sup>	0.10	1.94	2.75
ile	1.12 <sup>a</sup>	1.24 <sup>ab</sup>	1.35 <sup>b</sup>	0.04	1.07	1.43	2.05 <sup>a</sup>	1.65 <sup>b</sup>	1.64 <sup>b</sup>	0.07	1.58	2.15
leu	2.27 <sup>a</sup>	2.54 <sup>ab</sup>	2.90 <sup>b</sup>	0.10	2.18	3.07	4.32 <sup>a</sup>	3.52 <sup>b</sup>	3.44 <sup>b</sup>	0.15	3.30	4.54
tyr	1.00 <sup>a</sup>	1.22 <sup>b</sup>	1.38 <sup>bf</sup>	0.06	0.96	1.46	2.02 <sup>a</sup>	1.66 <sup>b</sup>	1.48 <sup>bf</sup>	0.08	1.42	2.12
phe	2.14 <sup>a</sup>	2.76 <sup>b</sup>	2.75 <sup>b</sup>	0.11	2.05	2.92	4.23 <sup>a</sup>	4.49 <sup>a</sup>	3.10 <sup>b</sup>	0.22	2.97	4.62
his	1.06 <sup>a</sup>	1.31 <sup>b</sup>	1.17 <sup>ab</sup>	0.04	1.02	1.39	1.93 <sup>a</sup>	2.12 <sup>b</sup>	1.33 <sup>c</sup>	0.12	1.28	2.18
lys	1.81 <sup>a</sup>	2.19 <sup>b</sup>	2.33 <sup>b</sup>	0.08	1.74	2.47	3.41 <sup>a</sup>	2.91 <sup>b</sup>	2.68 <sup>b</sup>	0.11	2.57	3.58
arg	2.31 <sup>a</sup>	3.84 <sup>b</sup>	3.81 <sup>b</sup>	0.26	2.22	4.07	7.25 <sup>a</sup>	7.69 <sup>a</sup>	3.16 <sup>b</sup>	0.72	3.04	7.92
cysH	0.72	0.78	0.76	0.01	0.69	0.82	1.23 <sup>a</sup>	0.98 <sup>b</sup>	0.96 <sup>b</sup>	0.05	0.92	1.29
metS	0.58 <sup>a</sup>	0.77 <sup>b</sup>	0.90 <sup>c</sup>	0.05	0.56	0.96	1.36 <sup>a</sup>	1.04 <sup>b</sup>	1.08 <sup>b</sup>	0.05	1.01	1.43

abbreviations: AA: aminoacid, AT: Austria, SK: Slovakia, GV: Green Veltliner, PB: Pinot Blanc, ZG: Zweigelt, SE: standard error of mean, Min: minimal value, Max: maximal value, asp: aspartic acid, thr: threonine, ser: serine, glu: glutamic acid, pro: proline, gly: glycine, ala: alanine, val: valine, ile: isoleucine, leu: leucine, tyr: tyrosine, phe: phenylalanine, his: histidine, lys: lysine, arg: arginine, cysH: cysteine, metS: methionine. The letters in superscripts indicates statistically significance of differences at the level 0.05 (Tukey test). Differences between countries within the varieties are marked by \* in superscripts at the level 0.05 (Independent samples T-test).

**DISCUSSION**

Igartuburu et al. (1991) reported similar amino acid content of wine industry by-products in comparison with oilseeds and cereals. Furthermore, Zhou et al. (2011) examined that wine industry by-products had specific functional properties because of their solubility and emulsifying activity. The most represented amino acids in grape by-products are glycine, glutamine acid and aspartic that was also reported in the research of Gazzola et al. (2014). Kamel et al. (1985), Zhou et al. (2011) and Mchedluri et al. (2014) found higher content of leucine (4.88, 4.80 respectively 5.34 g.kg<sup>-1</sup>) in grape products compared to whole grape bunch except of the PB variety from SK. Zhou et al. (2011) and Mchedluri et al. (2014) found higher content (5.83 and 11.65 g.kg<sup>-1</sup>) of aspartic acid in wine by-products samples in comparison with bunch and grape stem except of PB variety from SK. However, Kamel et al. (1985) determined lower content of aspartic acid (2.87 g.kg<sup>-1</sup>). On the other side, content of aspartic acid in grape pomace was in listed interval of Zhou et al. (2011) and Mchedluri et al. (2014) except of ZG variety from SK. In the case of threonine content, Turcu et al. (2018) determined higher concentrations (4.0 - 6.6 g.kg<sup>-1</sup>) in grape by-products compared to our findings. On the other side, Mchedluri et al. (2014) determined higher concentrations of threonine in grape by-products (9.77 g.kg<sup>-1</sup>). Zhou et al. (2011) determined the content of serine 3.37 g.kg<sup>-1</sup> in grape by-products. Higher content of serine was found only in bunch in PB variety from SK and in grape pomace in ZG from AT and PB from SK. Then, Turcu et al. (2018) observed very high content of glutamic acid from 23.3 to 35.2 g.kg<sup>-1</sup> in grape by-products samples compared to our results. However, Zhou et al. (2011) and Mchedluri et al. (2014) determined similar content of glutamic acid from 8.05 to 17.30 g.kg<sup>-1</sup>. But the content of glutamic acid tends to be lower in the bunch and stem. The content of proline was highest in the grape pomace, but Zhou et al. (2011) et al. found lower concentrations of proline (2.59 g.kg<sup>-1</sup>) compared to our results. Then, Mchedluri et al. (2014) determined similar content of proline 4.80 g.kg<sup>-1</sup> in comparison with grape stem. On the other side, the glycine content in Zhou et al. (2011) 8.68 g.kg<sup>-1</sup>, Mchedluri et al. (2014) 7.49 g.kg<sup>-1</sup> and Turcu et al. (2018) 6.0 - 10.8 g.kg<sup>-1</sup> was higher in comparison with analysed by-products. Also, higher content of alanine found Mchedluri et al. (2014) 7.48 g.kg<sup>-1</sup> in analysed grape by-products. Similar alanine content in grape by-products found Turcu et al. (2018) from 5.1 to 6.9 g.kg<sup>-1</sup>. The concentrations of valine were higher in comparison with Zhou et al. (2011) (3.82 g.kg<sup>-1</sup>) but only in SK PB whole bunch and AT ZG grape pomace. However, El-Shami et al. (1992) and Turcu et al. (2018) determined valine content up to 7.00 and 8.51 g.kg<sup>-1</sup>. Also, Turcu et al. (2018) found in grape by-products higher content of isoleucine from 4.40 to 5.60 g.kg<sup>-1</sup> in comparison with our samples. However, Mchedluri et al. (2014) did not detected isoleucine in grape by-products. Then, Leucine concentrations were lower compared to Zhou et al. (2011) (4.80 g.kg<sup>-1</sup>) except of whole bunch PB SK variety and ZG AT grape pomace. On the other side, higher content of leucine that was closer to these samples found Mchedluri et al. (2014) (5.34 g.kg<sup>-1</sup>). The tyrosine concentrations from 1.90 to 2.90 g.kg<sup>-1</sup> in Turcu et al. (2018) were almost the same in comparison with our research except of ZG grape pomace variety from AT. After that, all analysed samples had lower concentrations of phenylalanine compared to Mchedluri et al. (2014) where the content of phenylalanine up to 8.76 g.kg<sup>-1</sup> was observed. But Zhou et al. (2011) determined lower concentrations of phenylalanine in grape by-products (2.20 g.kg<sup>-1</sup>). Nevertheless, the most of

samples were higher content of phenylalanine than this value. Furthermore, the content of histidine in grape by-products were in the interval of Zhou et al. (2011) from 0.39 g.kg<sup>-1</sup> to 4.23 g.kg<sup>-1</sup> determined by El-Shami et al. (1992). After that, Zhou et al. (2011) found in grape by-products the content of lysine 0.65 g.kg<sup>-1</sup> that was close to the lowest content of lysine in our research. On the other side Turcu et al. (2018) found the concentrations of lysine up to 5.80 g.kg<sup>-1</sup>. However, it was lower in comparison with our upper content of lysine determined in ZG grape pomace from AT. Then, in the case of arginine content, El-Shami et al. (1992) found higher arginine concentrations (9.84 g.kg<sup>-1</sup>) in comparison with all samples. Moreover, Turcu et al. (2018) determined the arginine content in grape by-products up to 11.90 g.kg<sup>-1</sup>. Zhou et al. (2011) determined lower content of cysteine (0.39 g.kg<sup>-1</sup>) in comparison with analysed samples in our research and Mchedluri et al. (2014) did not find cysteine in grape by-products. On the other hand, Turcu et al. (2018) observed the content of cysteine in grape by-products from 1.50 to 2.40 g.kg<sup>-1</sup>. Finally, Mchedluri et al. (2014) determined higher concentrations of methionine 6.15 g.kg<sup>-1</sup>. In contrary, Zhou et al. (2011) found the content of methionine in grape by-products only 0.19 g.kg<sup>-1</sup>.

**CONCLUSION**

After the amino acid analysis in grape bunch the highest content of glutamic and aspartic acid was found. Then, similar pattern in amino acids with the lowest content between the countries and varieties was observed. However, the grape pomace amino acid content is highly variable and statistically different between the varieties and countries. In grape pomace, the highest content of glutamic, aspartic acid and arginine was observed. After that again, similar pattern in both countries in the term of the lowest amino acid content was observed. Thus, in the grape pomace low content of Cysteine, Methionine, Histidine and Proline was found. In comparison with whole grape bunch and pomace lower variability in amino acids content between the varieties was found in grape stem. But similarly like in grape bunch and stem, the highest content from amino acids was represented by aspartic, glutamic acid and arginine.

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