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SUGAR AND ORGANIC ACID PROFILES OF THE TRADITIONAL AND INTERNATIONAL APPLE CULTIVARS FOR PROCESSING

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Abstract

Current consumer trends require food products with „healthy image". This has led to an increased interest in traditional fruit cultivars and related products. Traditional apple cultivars in Bosnia and Herzegovina are a valuable source of desirable genetic characteristics including important pomological, nutritional and technological characteristics of the fruit. The sugar and organic acid profile of fruit is an important component of chemical composition and provides valuable information regarding the authenticity of fruit products. They also have an effect on the sensory properties and nutritional value of fruit products.

Ten traditional and two international commercial apple cultivars were analyzed, using HPLC, for individual sugars (sucrose, glucose, fructose and sorbitol) and individual organic acids (malic, citric, shikimic and fumaric acids).

Fructose was the most abundant among sugars that were quantified in apple fruit, followed by glucose, sucrose and sorbitol. Malic acid was predominated among the individual organic acids in both traditional and international apple cultivars, followed by citric, fumaric and shikimic acid. It was also found that cultivar had a significant influence on individual sugars and organic acid in the fruit.

Generally, traditional apple cultivars had higher sugar content and lower organic acid in relation to international ones and with respect of this they are sweet and good for direct consumption and "pekmez" production.

***Key words**: Apple cultivar, Individual sugars, Organic acids.*

1. Introduction

Consumption of fresh apple is often replaced by the intake of apple juice, due to their convenience and ability to quench thirst. In Europe, apple juice is a highly-consumed product, in second place after orange juice (Kay-Shuttleworth [1]). The quality of apple juice depends strongly on the apple cultivar used for processing (Eisele and Drake [2]). In recent years, the range of apple cultivars in the supermarkets has dramatically decreased. From integrated cultivation, small number of apple cultivars (e.g. Gala, Idared, Granny Smith) is sold all over Europe. In contrast to this small number, there are a lot of traditional, old apple cultivars even in a country as small as Bosnia and Herzegovina which offer a wide range in sugar, acid and phenol content (Begic-Akagic *et al*. [3]). Few literature data are available on the on the nutrition properties of little widespread traditional apple cultivars. Such lack of information prevents exploitation of these germplasms for genetic improvement of new cultivars and for the re-evaluation of local food products, which may attract a large share of consumers oriented towards natural food evoking ancient flavours. Some apple cultivars such as traditional ones are scab-resistant and require a reduced number of sprays during fruit growth, thereby decreasing production costs and pesticide residues in the fruit and related products. Each cultivar of fruit has its own significant chemical composition; however, differences in compound amounts may occur depending on the maturity, environmental factors (Sturm and Stampar [4]; Markuszewski and Kopytowski [5]), horticultural practices applied in an orchard (Arakawa *et al*. [6]; Yuan and Greene [7]; Weibel *et al*. [8]; Veberic and Stampar [9]; Peck *et al*. [10]; Amarante *et al*. [11]) storage conditions (Roth *et al*. [12]; Begić-Akagić *et al*. [3]).

Among the most important constituents of apple and related products are sugars and acids (Suni *et al*., [13]; Markowski *et al*. [14]; Nour *et al*. [15]; Carvalno and Chiquetto [16]; Gastol *et al*. [17]). Many researchers (Sturm and Stampar [4]; Zhang *et al*. [18]) have reported that apple fruits contain fructose, glucose, sucrose and sorbitol. Among organic acids, apple fruits contain malic, citric, shikimic, fumaric and also quinic acid (Campeanu *et al.* [19]).The sugar profile of fruit pulp is an important component of chemical composition and provides valuable information regarding the authenticity of fruit juices (Wu *et al.* [20]). It also has an effect on the sensory properties and nutritional value of fruit products. The content of organic acids in fruit juices not only influences their flavour but also their stability, nutrition, acceptability and keeping quality. Besides their importance in flavour, acids are important in gelling product processing because they affect the gelling properties of pectin. The sugar/acid ratio is responsible for the taste and flavour of apples (Mikulic Petkovsek *et al.* [21], and Wu *et al.* [20]).

To the best of our knowledge, sugars and organic acid composition of apple fruits grown in Bosnia and Herzegovina have no investigated until now. Some of these cultivars may be suitable for apple juice production and other ones for table consumption and for other products such as 'pekmez' production. Therefore the aim of the present study was i) to compare the sugar and acid content of traditional and international apple cultivars ii) to identify apple cultivars the most suitable for processing into different fruit products, according to sugar/acid ratio.

2. Materials and Methods

2.1 Materials

The analyzed cultivars were obtained from the ex-situ collection orchard in Srebrenik (Bosnia and Herzegovina). Fruit production in the mentioned orchard is done according to the principles of integrated pest management (IPM). These production principles define the use of chemicals according to precise guidelines and restrictions, especially for the protection of the plants against pests and diseases. The trees were grafted on the MM 106 rootstock and the trees were planted in year 2002 (training system was spindle with planting distance of 4.0 x 2.0 m). Fruit sampling was performed at technological maturity, which was determined using the starch iodine test. Fruit from all apple cultivars were picked from the central part of the canopy (outer layer of the branches). Overall twelve apple cultivars were analysed in this study, ten traditional ('Prijedorska Zelenika'; 'Kanjiška'; 'Paradija'; 'Senabija'; 'Žuja'; 'Ljepocvjetka'; 'Funtača'; 'Masnjača'and 'Dobrić') and two international ('Red Delicious' and 'Granny Smith').The analyzed traditional cultivars have previously been genetically characterized using molecular markers (Gasi *et al.* [22]), and were determined to be a representative of the traditional B&H apple germplasm.

<u>2.1.1 Standards and chemicals</u>

For the quantification of the organic acid (citric, fumaric and shikimic acids) as well as individual sugars (fructose, sucrose, glucose and sorbitol) standards acquired from Fluka (Buchs, Switzerland) were used. Malic acid was obtained from Merck (Darmstadt, Germany).

2.2 Methods

<u>2.2.1 Sugars and organic acids extraction and analysis</u>

For the analysis of individual sugars and organic acids in fresh fruit the samples were transferred on dry ice to the laboratory of Chair for Fruit Growing of Department of Agronomy at the Biotechnical Faculty, Ljubljana, Slovenia. Sugars and organic acids were extracted from the whole fruit. All the analyses were performed in five replications were made (n = 5) for every cultivar. Each repetition consisted of analyses on 15 apples sampled from five trees. Ten grams of each sample were brought to 40 mL of total volume with bi-distilled water and homogenized for one min at 24 ^{0}C with a T-25 Ultra-Turrax (IKA - Labortechnik, Staufen, Germany). Samples were left to extract for half an hour at 24 ^{0}C and centrifuged at 10,000 rpm for 7 min. at 5 ^{0}C (Eppendort 5810 R Centrifuge, Hamburg, Germany). The supernatant was used for analysis after filtration through a 0.45 µm cellulose filter (Macherey - Nagel, Düren, Germany) into vials. The Thermo Separation Products HPLC system (Riviera Beach, FL; Pump model P1000; auto sampler model AS1000) was used for the analysis of individual sugar content (sucrose, glucose, fructose and sorbitol) and individual organic acid content (malic, citric, shikimic and fumaric acids). Injection volume of sample was 20 µl. The separation of sugars was carried out using a Rezex RCM - monosaccharide column (300 x 7.8 mm, Phenomenex, USA), with the temperature maintained at 65 ^{0}C at a flow rate of 0.6 mL min^{-1} . The isocratic method with bi-distilled water as mobile phase was used. Refractive index (RI) detector was used for monitoring the eluted carbohydrates according to Hudina and Stampar [23]. Organic acids were analysed using the Razex ROA - Organic Acid H+ (300 x 7.8 mm, 8 µm; BioRad, USA) with the same conditions previously described for sugars analysis. As a mobile phase, 4 mM sulphuric acid was used. UV detector was used for detection with a wavelength set at 210 nm. The content of individual organic acids levels (malic, citric, shikimic and fumaric acid) as well sugars (sucrose, fructose, glucose and sorbitol) were expressed in g kg^{-1} of fresh weight (FW). Total acids and sugars were calculated as the sum of individual acids as well sugars. We used values of total sugar content and total organic acid content to calculate the sugar/organic acid ratio.

<u>2.2.2 Statistical analysis</u>

Data were reported as the mean ± standard error of five replicates. The results were compared by one-way analysis of variance (ANOVA) and the determined differences were tested by Tukey test at a significance level of 0.05 (using the SPSS 16 program). The chemical data (content of individual and total sugar and acids, sugar/acid ratio and G/F ratio) was analyzed using PCA to identify any factors differentiating the apple cultivars. All statistical analyses were done using StatBox 6.7 Grimmer Soft, France statistical computer program.

3. Results and Discussion

The content of sugars and organic acids depend on the plant genotype (Mikulic Petrovsek *et. al*. [21]); Wu *et. al.* [20]) and is also influenced by environmental factors and by horticultural practice undertaken in an orchard (Hudina and Stampar [23]).The results for individual sugars of the apples from the different cultivars are given in Table 1.

A great variation in terms of individual and total sugars was observed among the apple cultivars and the differences were statistically significant (Tab.1). Regarding these, since all twelve apple cultivars used in this study were grown in the same location using similar horticultural practices, the variation in individual and total sugars demonstrates that the genetic variability led to the differences in the synthesis of sugars in these apple selections. Total sugar content of the apple cultivars ranged between 80.06 to 119.7 gkg^{-1}. Among the single components, fructose and glucose were identified as major items. All analyzed contain more fructose and less glucose, a fact that is positive for diabetes patients, since it keeps the blood-sugar level constant (Hecke *et al*. [24]).

As shown in Table 1, the fructose content of 'PZ' apple (60.15 gkg^{-1}) is the highest of all the fructose concentrations of the other cultivars. Glucose concentration ranged between 20.37 and 43.49 gkg^{-1}. The highest amount of sucrose was observed in 'ZU' (24.52 gkg^{-1}) while the lowest was in 'GS' apple (8.73 gkg^{-1}).

Average fructose and glucose level for analyzed apple cultivars was 52.39 and 33.41 gkg^{-1}and was in agreement with those reported by Wu *et al*. [20]. The same authors reported the higher average sucrose content than obtained in this study (16.01 gkg^{-1} FW). Sorbitol was present in small amount since only 0.022 gkg^{-1} FW. The highest contents were measured by cultivars ''FU' and 'RD' (0.174 gkg^{-1} FW). Contents of sorbitol were lower in all analyzed apple cultivars compared to those reported by Sturm and Stampar [4], Mikulic Petkovsek *et al*. [25] and Wang *et al*. [26]. Differences in glucose and fructose contents are reflected in the glucose/fructose ratio. The highest ratio (0.93) was found for 'DO' cultivar, while in 'GS' it was 0.40.

The total acid concentration of tested cultivars ranged between 0.62 ('PZ') and 15.36 g kg^{-1}fresh weight ('Granny Smith'). There were significant differences between analyzed cultivars in total acid and individual organic acids. The major organic acids were malic, fumaric, citric and shikimic acids (Table 2). The predominant organic acid in the tested apple cultivars was malic acid. Fumaric acid concentrations ranged between 0.04 ('PZ') and 5.20. The highest concentration was found in Granny Smith (7.56 g kg^{-1}FW) and the lowest in 'MA' (0.41 g kg^{-1}FW). For the cultivars tested, citric acid content varied between 0.04 ('PZ') and 2.59 g kg^{-1}FW ('GS'). Shikimic acid was present in lowest quantities compared to other analysed acids. The highest content was measured in cultivar 'ZU' while another analyzed cultivars contained from 0.01 to 0.13g kg^{-1} FW. The sugar/acid ratio of the tested cultivars were in range from 5.36 ('GS') to 189.90 ('PZ'). According to research made by Lea [27] apple cultivars with sugar/acid rations below 20 are acidic and are suitable for processing and cider production, while cultivars with sugar/acid rations higher than this value are sweet, and good for direct consumption.

Table 1. The content of individual sugars in different apple cultivars (g kg^{-1}fresh weight). Each mean is the average of 5 replicates

Sugars	Cultivars											
	PZ	KA	PA	SR	SE	ZU	LJE	FU	MA	DO	RD	GS
Fructose	60.15 ± 0.811a	34.93 ± 2.045e	53.29 ± 1.330bc	50.80 ± 1.016cd	53.15 ± 1.469bc	55.41 ± 0.438bc	57.75 ± 0.954ab	52.80 ± 0.550bc	54.13 ± 0.739bc	46.73 ± 0.503d	57.21 ± 1.556ab	52.38 ± 1.080c
Glucose	34.97 ± 0.220c	29.78 ± 0.050d	30.20 ± 1.004d	38.73 ± 0.024b	29.80 ± 0.260d	39.68 ± 0.722b	39.89 ± 0.008b	43.35 ± 0.028a	20.37 ± 0.734e	43.49 ± 0.197a	29.57 ± 0.130d	21.15 ± 1.069e
Sucrose	22.69 ± 0.253b	15.28 ± 0.210f	19.59 ± 0.340d	12.79 ± 0.484h	9.05 ± 0.188i	24.52 ± 0.469a	12.68 ± 0.171h	21.33 ± 0.573c	14.21 ± 0.515fg	13.11 ± 0.278gh	18.10 ± 0.390e	8.73 ± 0.324i
Sorbitol	0.022 ± 0.002e	0.064 ± 0.008d	0.050 ± 0.004d	0.111 ± 0.006c	0.056 ± 0.003d	0.147 ± 0.003 d	0.068 ± 0.002d	0.174 ± 0.008a	0.151 ± 0.005b	0.097 ± 0.005c	0.174 ± 0.012a	0.047 ± 0.005d
Total sugar	117.82a	80.06e	103.1bc	102.4cd	92.06bc	119.7bc	110.39b	117.6bc	88.86bc	103.4d	105.05b	82.31c
G/F ratio	0.58	0.85	0.57	0.76	0.56	0.72	0.69	0.82	0.38	0.93	0.52	0.40

Different letters in rows from a to i for each sugar indicate significantly different values among cultivars at $p < 0.05$.
PZ - Prijedorska zelenika; KA - Kanjiska; PA - Paradija; SE - Senabija; ZU - Zuja; LJE - Ljepocvjetka; FU - Funtaca; MA - Masnjaca; DO - Dobric; RD - Red Delicious; RS - Granny Smith.

Table 2.Content of individual organic acids in different apple cultivars (g kg^{-1}fresh weight). Each mean is the average of 5 replicates

Organic acid	Cultivars											
	KA	PA	MA	ZU	DO	SR	SE	PZ	LJE	FU	RD	GS
Malic acid	4.43 ± 0.247c	1.95 ± 0.021d	0.41 ± 0.011g	1.41 ± 0.025e	1.18 ± 0.038e	0.76 ± 0.046f	0.58 ± 0.018fg	0.51± 0.020fg	0.79 ± 0.049f	5.61 ± 0.114b	1.20 ± 0.021e	7.56 ± 0.071a
Citric acid	0.45 ± 0.018de	0.96 ± 0.022c	0.10 ± 0.001f	0.66 ± 0.034cd	0.77 ± 0.050cd	0.19 ±0.001ef	0.28 ± 0.001ef	0.04 ± 0.001f	0.26 ± 0.001ef	1.87 ± 0.017b	0.67 ± 0.033cd	2.59 ± 0.097a
Shikimic acid	0.03 ± 0.002g	0.11 ±0.004c	0.09 ± 0.00d	0.16 ± 0.003a	0.06 ± 0.003e	0.09 ± 0.001d	0.01 ± 0.00h	0.03 ± 0.003fg	0.04 ± 0.005f	0.13 ± 0.003b	0.10 ± 0.00c	0.02 ± 0.00h
Fumaric acid	1.87 ± 0.024c	1.30 ± 0.016d	0.027 ± 0.004f	0.75 ± 0.008e	0.70 ± 0.011e	0.16 ± 0.002f	0.18 ± 0.006f	0.04 ± 0.001f	0.11 ± 0.000f	2.87 ± 0.021b	0.09 ± 0.001e	5.20 ± 0.037a
Total acid	6.78c	4.32d	0.63g	2.97e	2.71e	1.20f	1.04f	0.62g	1.20f	10.49b	2.92e	15.36a
Sugar /acid ratio	11.81	23.86	141.45	40.31	38.22	85.33	88.59	189.90	92.04	11.22	36.01	5.36

Different letters in rows from a to i for each acids indicate significantly different values among cultivars at $p < 0.05$.
PZ - Prijedorska zelenika; KA - Kanjiska; PA - Paradija; SE - Senabija; ZU - Zuja; LJE - Ljepocvjetka; FU - Funtaca; MA - Masnjaca; DO - Dobric; RD - Red Delicious; RS - Granny Smith.

As shown in Table 2, all analyzed cultivars had sugar/acid rations higher than 20, being classified as sweet except 'GS', 'FU', and 'KA'. Regarding that analyzed cultivars are classified as sweet could be used for direct consumption or for 'pekmez' processing while cultivars such as 'GS', 'FU', and 'KA' for juice processing. According to Wu *et al*. [20] for the production of apple juice concentrates with good quality, 'Granny Smith' and 'Ralls' are ideal cultivars because they had relatively high contents of soluble solids and acids. 'Granny Smith' had the high levels of total acids (7,3gL^{-1}) and the lowest sugar/acid ratio (12,84) of the eight apple cultivars followed by 'Ralls' (24,12). Paganini *et al*. [28], Nogueira *et al*. [29] and Vieira *et al*. [30], found that different apple cultivars grown in Brazil had rations from 31.5 to 66.80. Obtained results in our study for total acids are not in agreement with Hecke *et a*l. [24] who reported values of total acids between 6.26 and 17.85 g kg^{-1} FW except 'KA', 'FU' and 'GS'. The most of analyzed cultivars had lower total acid content than that reported. The possible reason could be related to the specific geographical nature of the different areas and different agronomical practices. Hecke *et al*. [24] reported lower data for apple cultivars with shikimic acid content between 0.002 g kg^{-1} FW and 0.057 g kg^{-1} FW. Shui and Leong [31] reported that the low content of fumaric acid in apple juice indicates its authenticity and good quality. The same authors reported that fumaric acid content of 10 mg L^{-1} in apple juice could only be due to the addition of synthetic malic acid or a microbial spoilage due to the presence of *Rhizopus stolonifer*. The obtained results for total and individual sugars and acids level of the apple cultivars in this study demonstrated that the twelve cultivars are different in analyzed components. In addition, these parameters vary greatly among the studies cited and among the regions considered by the authors. This may be due to the specific geographical nature of the different areas and different agronomical practice. Regarding these, the differences in apple cultivars in terms of analyzed components is attributed to the origin of the plant material (Begic-Akagi *et al*. [32]; Panzella *et al*. [33]) since analyzed cultivars were grown under the same geographical conditions and with the same applied horticultural practices.

Inspection of the Fig. 1 showed that 'Masnjaca','Red Delicius' were associated with the content of fructose whereas 'Funtaca' was mainly associated with the content of sucrose and consequently with total sugar content. Cultivars such as 'Zuja' and 'Prijedorska zelenika' belong to both groups that mean these two apple cultivars were determined by high content of fructose, sucrose and consequently total sugar content. In the negative part of PC 2 'Dobric' and 'Kanjiska' were sited and they were associated with high value of glucose/fructose ratio.

Fig. 2 shows the PCA plots of 12 analyzed apple cultivars based on acid content and sugar/acid ratio. Differences were observed between 'Funtaca'/'Granny Smith' and 'Masnjaca'/'Prijedorska zelenika'/'Senabija' among PC1. The acids responsible for the separation were citric, malic, fumaric and consequently total acid content, mainly associated with 'Funtaca' and 'Granny Smith', while high sugar/acid ratio was associated with 'Masnjaca', 'Prijedorska zelenika' and 'Senabija'. The content of shikimic acid was the basis for the separation of 'Zuja'. This apple cultivar was determined by higher content of shikimic acid. Keenan *et al*. [34] were investigated interrelationships between the parameters analysed different cultivars by multivariate analyses.

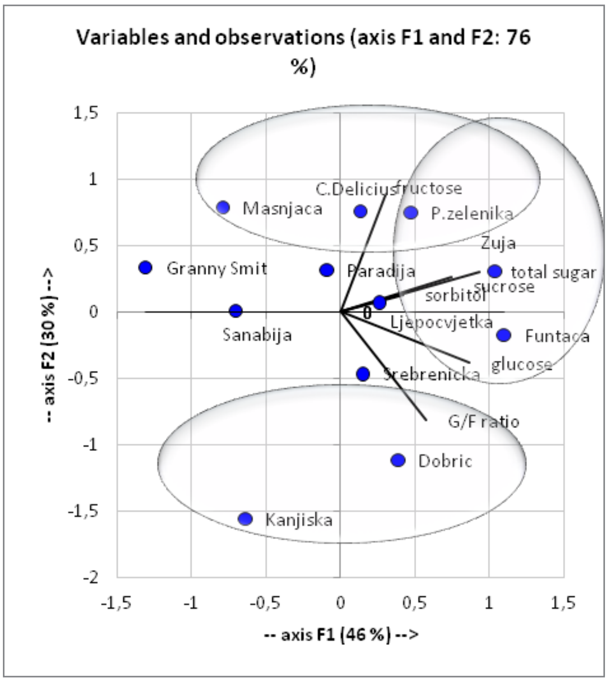

Figure 1. PCA plot presenting graphical representation of the position of each apple cultivar in relation to the individual and total sugar content and glucose/fructose ratio (G/F ratio)

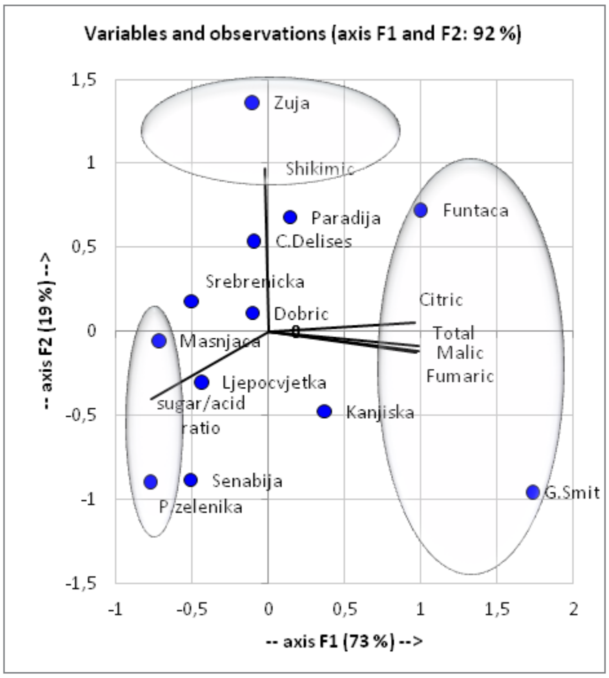

Figure 2. PCA plot presenting graphical representation of the position of each apple cultivar in relation to the individual and total acid content and sugar/acid ratio

4. Conclusions

- The results describe above testified to the great differences in the composition of different apple cultivars, and suggested that the total and individual sugar and acids are the most important factors for the characterisation of apple cultivars with respect to their nutritional value, potential use for different products and indicate their authenticity.

- Amongst the twelve apple cultivars investigated, the best for direct consumption are 'Zuja', 'Dobric' and 'Red Delicious', and for 'pekmez' processing are 'Masnjaca', 'Senabija', 'Prijedorska Zelenika' and 'Ljepocvjetka' with their high level of sugar/acid ratio.

- For the production of fruit juice and cider and gelling products, the best seemed to be 'Granny Smith', 'Funtaca', 'Kanjiska' and 'Paradija', with their relatively low sugar/acid ratio.

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