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Chemical comparison of 100% apple, orange and grapefruit juices directly pressed and made from concentrate

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Abstract: One hundred per cent apple, orange and grapefruit juices were analysed for ascorbic acid (AA) content, total polyphenolic content (TPC), and other parameters: titratable acids (TA), malic acid (MA), citric acid (CA), volatile acid (VA), soluble solids (SS), density, and the content of free amino acid as formol number (FN). Some apple juices on the Czech market contain added vitamin C, and the AA content of such juices (268–632 mg L^{-1}) is several times higher than that of apple juices without added vitamin C (around 50 mg L^{-1}). Therefore, the enriched apple juices had a vitamin C content comparable to citrus fruit juices (orange and grapefruit). All contents were compared separately for juices made from concentrate and for directly pressed juices. The effect of the production method was statistically significant ($P < 0.05$) only for TPC in apple juices, where it was major [473 ± 136 mg gallic acid equivalents (GAE) L^{-1} from concentrate *vs.* about twice the value of 798 ± 193 mg GAE L^{-1} in directly pressed juice], and only to a smaller extent for TA in grapefruit juices (12.5 ± 0.8 g L^{-1} from concentrate, which was about 30% more than in directly pressed juice).

Keywords: vitamin C; polyphenols; squeezed juices (100%); juices from concentrate (100%); fruits

Consumer demand for fruit juices is steadily increasing as clinical studies suggest that there are health benefits associated with fruit and juice consumption (Twohig et al. 2011). Fruit juices and fruit juice mixtures ('smoothies') are more convenient to consume and have a longer shelf life than fresh fruit (Nicklas et al. 2015).

Consumption of drinks that contain sugar is associated with gaining weight and obesity. Since 100% fruit juices have a similar sugar content to sweetened beverages, fruit juice consumption may also be associated with an increased risk of diabetes (Hägele et al. 2018). However, unlike sweetened beverages, juices contain important nutrients including vitamin C, potassium, folate, magnesium, β-carotene and flavonoids (Hägele et al. 2018). There are two types of fruit juices on the market, namely directly pressed juices and juices made from concentrate. Such juice is produced when water is evaporated from the juice and the resulting concentrate is diluted to the same composition as the original juice (Bhattacharjee et al. 2017). Directly pressed juice is produced by squeezing the juice from the fruit, which is packaged, pasteurised and dispatched for distribution. It usually has a shelf life of 1 week to 2 months (Ashurst et al. 2016). The most common

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heat treatment remains the most widely used method used to extend the shelf life of juices. However, it can negatively affect nutritional components such as anthocyanins, carotenoids, vitamins, and bioactive compounds (Van den Hout et al. 1999; Kechinski et al. 2010; Barros et al. 2011; Provesi et al. 2011), and sensory parameters such as colour, aroma, and taste (Timoumi et al. 2007; Nisha et al. 2009). Other types of treatments like high-pressure processing (HPP) or pulsed electric field (PEF) technology are increasingly used on an industrial scale (Olsen et al. 2010). Juices can be fortified with various substances, either vitamin C or extracts that increase antioxidant properties (Kulichová et al. 2018). The goal of this study is a chemical comparison of apple, orange and grapefruit juices on the Czech market including the type of production (directly pressed *vs.* from concentrate).

MATERIAL AND METHODS

Chemicals

The following chemicals were used for the analysis: monopotassium phosphate (KH_2PO_4) (99%; Penta, Czech Republic), L-ascorbic acid (L-AA) (99%; Sigma-Aldrich, Germany), malic acid (MA) (99%; Penta, Czech Republic), citric acid (CA) (99%; Sigma-Aldrich, US), sodium hydroxide (NaOH) (98%; Ivo Průdek, Czech Republic), gallic acid (98%; Sigma-Aldrich, US), Folin-Ciocalteau reagent (FCR) [practical grade (p.a.); Penta, Czech Republic], sodium carbonate (Na_2CO_3) (p.a.; Lach-Ner, Czech Republic), and deionised water.

Material

Commercial 100% fruit juices, both directly pressed (s) and those made from concentrate (c), were used in the analysis, with a total of 49 products purchased from retail outlets in the Czech Republic. They included apple (A), orange (O) and grapefruit (G) juices.

The number of directly pressed apple juices was 10 (As1–As10), of which 3 (As8–As10) were fortified by the producer with ascorbic acid (AA); 13 apple juices from concentrate (Ac1–Ac13), of which 1 (Ac13) was fortified by the producer with AA; 5 orange juices directly pressed (Os1–Os5); 15 orange juices from concentrate (Oc1–Oc15); 2 grapefruit juices directly pressed (Gs1 and Gs2); 4 grapefruit juices from concentrate (Gc1–Gc4).

Methods

High-performance liquid chromatography (HPLC) determination of AA, MA, and CA. Juice sample (2 mL) was diluted with water to the final volume of 10 mL. Using a nylon filter (0.22 µm, CHS FilterPure Nylon Syringe Filters; Chromservis, Czech Republic), the diluted sample was filtered into a brown vial. Conditions of chromatographic analysis: Prevail 5 µm Organic Acid 110Å high-performance liquid chromatography (HPLC) 250 × 4.6 mm column, flow rate of mobile phase 25 mM KH_2PO_4 1 mL min^{-1}, ultraviolet (UV) detection at 210 nm, column temperature +30 °C on HPLC instrument (ECP 2000; Ecom, Czech Republic), UV-visible (UV-VIS) detector (Sapphire 600; Ecom, Czech Republic). The determination was carried out by means of a calibration curve on standards L-AA [R^2 = 0.9997, limit of detection (LOD) = 3.7 mg L^{-1}, limit of quantification (LOQ) = 12.4 mg L^{-1}], MA (R^2 = = 0.9994, LOD = 5.4 mg L^{-1}, LOQ = 8.0 mg L^{-1}), and CA (R^2 = 0.9995, LOD = 4.1 mg L^{-1}, LOQ = 13.6 mg L^{-1}). The same method was used in the previous study (Šnurkovič 2013).

Titratable acids (TA) and volatile acids (VA). Titratable acids (TA) were determined by potentiometric titration using a combination electrode (SenTix 81; WTW, Germany), where 10 mL of juice were titrated with a 0.1 M NaOH solution to pH 8.1 by Official Method 940.15 (Latimer 2019). Volatile acids (VA) were determined in the same way as TA after distillation of the juices by Official Method 964.08 (Latimer 2019).

Total polyphenol content (TPC). This method is based on spectrophotometric measurement of colour products occurring during the reaction of hydroxyl groups of phenolic compounds with FCR. Sample preparation: the juice sample (0.1 mL) was pipetted into a 50 mL volumetric flask and mixed with 20 mL of distilled water and 1 mL of FCR. After 3 min, 5 mL of 20% Na_2CO_3 solution was added, the volumetric flask was filled with distilled water to the mark and stirred. Thirty minutes later, the absorbance was measured in a spectrophotometer (Specord 50 PLUS; Analytic Jena, Germany) at the wavelength of 700 nm using a 10-mm cuvette. The result was compared with the absorbance of a blind sample (1 mL of FCR, 5 mL of 20% Na_2CO_3 solution, and distilled water up to 50 mL). The content of total polyphenols was thereafter converted to the fresh plant matter and expressed as milligrams of gallic acid equivalent per 1 litre of juice (mg GAE L^{-1}). The same method was used in the previous study (Soural et al. 2019).

Determination of formol number (FN). The formol number (FN) expresses the total content of free amino acids (Rutherfurd 2010). Juice (25 mL) was neutralised with 0.1 M NaOH solution to pH 8.1. Formaldehyde solution (10 mL, 35%) was added to the sample at constant stir-

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ring (WiseStir MSH-20D; Witeg Labortechnik GmbH, Germany) and the sample was titrated with a volumetric NaOH solution to pH 8.1 [by pH-meter (inoLab pH 7310; WTW, Germany)]. FN corresponds to the consumption of 0.1 M NaOH in mL per 100 mL of sample.

Soluble solids (SS) and density. The soluble solids (SS) content was analysed using an Abbe refractometer (AR4; A.KRÜSS Optronic GmbH, Germany) by Official Method 932.14 (Latimer 2019). The results were expressed as SS content in degrees Brix (°Bx).

Density was measured pycnometrically at 20 °C. The density of the sample was calculated from the weight of the dry pycnometer, the pycnometer with water, and the sample by Official Method 945.06 (Latimer 2019).

Statistical method. Average values and standard deviations (SD) were calculated for all measurements from two replicates. Statistical data were analysed by analysis of variance (ANOVA), applying Tukey's multiple range test for making comparisons with Statistica Cz 12 and MS Excel 2010 software.

RESULTS AND DISCUSSION

AA. The vitamin C content (Table 1, Figure 1) ranged from 15 mg L^{-1} to 775 mg L^{-1}. The lowest values were observed in apple juices pressed without any vitamin C additions (*) (mean value of As* 41 ± 20 mg L^{-1}, of As7 only 15 ± 1 mg L^{-1}) and from concentrate without any vitamin C additions (mean value of Ac* 58 ± ± 19 mg L^{-1}). Varming et al. (2013) determined the average vitamin C content in apple juices from 71 apple cultivars to be 64 ± 27 mg L^{-1}, which coincides with our measured values. Four apple juice samples (As8–As10 and Ac13) which were not included in the averages of As* and Ac* due to the addition of AA by the manufacturer contained comparable amounts of vitamin C (mean value 479 ± 153 mg L^{-1}) as the orange (Os 397 ± 134 mg L^{-1} and Oc 358 ± 123 mg L^{-1}) and grapefruit juices (Gs 443 ± 81 mg L^{-1} and Gc 248 ± ± 37 mg L^{-1}). When apple juices with vitamin C additions were excluded, the apple juices without any additions (As* and Ac*) were statistically different from the orange and grapefruit juices (Os, Oc, Gs, and Gc); conversely, including the apple juices with vitamin C additions (As and Ac), only the apple juice from concentrate (Ac) was statistically different from all others (As, Os, Oc, Gs, and Gc). Meléndes-Martínez et al. (2007) determined vitamin C contents ranging from 333 mg L^{-1} to 441 mg L^{-1} in orange juices produced in Valencia. Within this interval were 7 of the 20 orange juices analysed in this work, but the range of values was

Table 1. The average values of all measured apple (A), orange (O) and grapefruit (G) juices directly pressed (s) or made from concentrate (c) for the measured parameters

Compounds	As	Ac	Os	Oc	Gs	Gc
TA (g L^{-1})	5.24 ± 1.94^{b} $*5.45 \pm 2.31^{/bc}$	4.98 ± 0.58^{b} $*4.98 \pm 0.61^{/b}$	$7.13 \pm 0.49^{a/ac}$	$7.85 \pm 0.48^{a/a}$	$9.66 \pm 1.36^{a/a}$	$12.50 \pm 0.80^{c/d}$
AA (mg L^{-1})	182 ± 212^{ab} $*41 \pm 20^{/b}$	94 ± 124^{b} $*58 \pm 19^{/b}$	$397 \pm 134^{a/a}$	$358 \pm 123^{a/a}$	$443 \pm 81^{a/a}$	$248 \pm 37^{ab/a}$
MA (g L^{-1})	6.02 ± 1.93^{b}	6.87 ± 0.81^{b}	1.48 ± 0.49^{a}	0.99 ± 0.29^{a}	0.20 ± 0.07^{a}	0.26 ± 0.05^{a}
CA (g L^{-1})	0.12 ± 0.06^{a}	0.11 ± 0.06^{a}	8.95 ± 1.49^{b}	9.54 ± 1.67^{b}	15.95 ± 0.78^{c}	14.19 ± 1.83^{c}
VA (g L^{-1})	0.09 ± 0.05^{b}	0.06 ± 0.04^{b}	0.13 ± 0.06^{c}	0.09 ± 0.04^{ac}	0.06 ± 0.04^{a}	0.05 ± 0.02^{a}
TPC (mg L^{-1})	798 ± 193^{a}	473 ± 136^{b}	859 ± 78^{a}	$1\,031 \pm 270^{a}$	$1\,092 \pm 159^{a}$	$1\,016 \pm 164^{a}$
FN (mL 0.1 M NaOH)	4.81 ± 1.53^{b}	4.17 ± 0.63^{b}	23.51 ± 1.69^{c}	20.24 ± 4.27^{ac}	15.93 ± 0.98^{a}	17.51 ± 0.43^{a}
SS (°Bx)	11.30 ± 0.43^{a}	11.37 ± 0.38^{a}	11.24 ± 0.27^{ab}	11.34 ± 0.48^{a}	10.93 ± 0.83^{ab}	10.33 ± 0.57^{b}
Density (g mL^{-1})	1.0467 ± 0.0017^{a}	1.0452 ± 0.0008^{a}	1.0454 ± 0.0018^{a}	1.0459 ± 0.0015^{a}	1.0437 ± 0.0048^{ab}	1.0402 ± 0.0012^{b}

[a-d]Different letter for each parameter indicate the significant differences ($P = 0.05$); *juices without vitamin C addition; TA – titratable acid; AA – ascorbic acid; MA – malic acid; CA – citric acid; VA – volatile acids; TPC – total polyphenolic content; FN – formol number; SS – soluble solid

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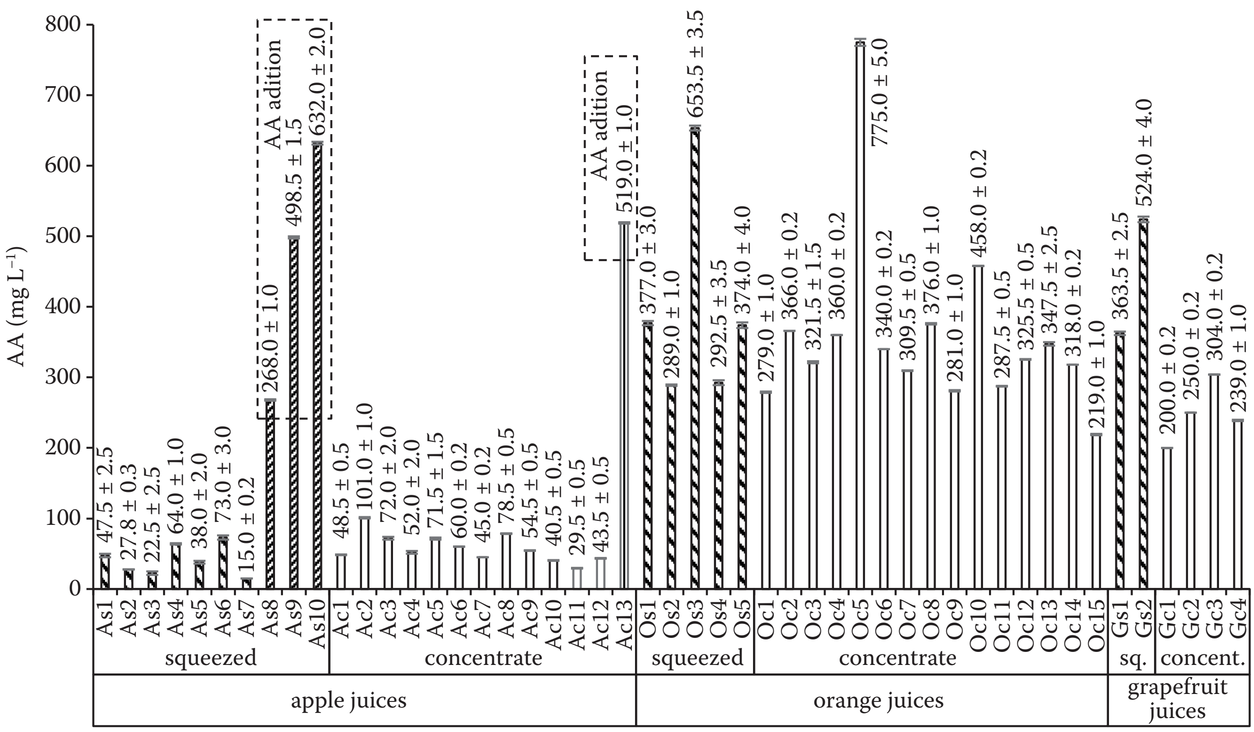

Figure 1. The content of ascorbic acid (AA) in 100% apple (A), orange (O) and grapefruit (G) juices

Pressed (s) (As8–As10) and concentrate (c) (Ac13) apple juices were fortified with vitamin C by the producers and are marked with different shading and marked with dashed rectangles

219–775 mg L^{-1} for all 20 juices. Our measured values and the literature (Meléndes-Martínez et al. 2007; Varming et al. 2013) suggest that apple juices have a lower vitamin C content than citrus fruits juices (oranges and grapefruits).

TA. The content of TA (Table 1, Figure 2) was in the range of 3.09 g L^{-1} to 13.03 g L^{-1} in the individual analysed beverages. Grapefruit juices made from concentrate had the highest acid content (Gc 12.50 ± 0.80 g L^{-1}, and Gc4 even had 13.03 g L^{-1}). The lowest contents were measured in apple juices (Ac 4.98 ± 0.58 g L^{-1} and As 5.24 ± ± 1.94 g L^{-1}, when As2 had only 3.09 g L^{-1}). The addition of vitamin C to apple juices (As8–As10 and Ac13) did not show any increase in the mean value of TA concentration compared to apple juices without this addition (Ac 4.98 ± 0.58 g L^{-1} *vs.* Ac* 4.98 ± 0.61 g L^{-1} and As 5.24 ± 1.94 g L^{-1} *vs.* As* 5.45 ± 2.31 g L^{-1}). Statistically, both types of apple juice (As, Ac) differed from both types of orange juice (As 7.13 ± 0.49 g L^{-1}, Oc 7.85 ± 0.48 g L^{-1}) and directly pressed grapefruit juices (Gs 9.66 ± 1.36 g L^{-1}), while all juices were statistically different from grapefruit juice made from concentrate (Gc 12.50 ± 0.80 g L^{-1}). Rekha et al. (2012) determined the total acid content in the range of 5.6–10.7 g L^{-1} of juices produced from citrus fruits. For all 20 orange samples (Os and Oc) and both grapefruit directly pressed juices (Gs), the values measured by us were in the same range.

TPC. The values of polyphenols in the analysed juices ranged from 269 mg L^{-1} to 1 629 mg L^{-1} GAE (Table 1, Figure 3). The lowest values were found in apple juices made from concentrate (Ac 473 ± 136 mg L^{-1} GAE, with the lowest being 269 ± 21 mg L^{-1} GAE in sample Ac4). Virtually twice the amount of polyphenols was found in directly pressed juices (As 798 ± 193 mg L^{-1} GAE with the highest value of As10 1 181 ± 10 mg L^{-1} GAE). These values are consistent with Vrhovsek et al. (2004), where juices from eight apple cultivars were analysed with values ranging from 662 mg L^{-1} to 2 119 mg L^{-1} GAE (and/or mg kg^{-1}), and also with Pavun et al. (2018), where three apple 100% juices on the Serbian market had a TPC range of 256–442 mg L^{-1} GAE. Directly pressed orange juices (Os 859 ± 78 mg L^{-1} GAE) showed polyphenol levels similar to juices made from concentrate (Oc 1 031 ± 270 mg L^{-1} GAE), the lowest polyphenol content was in Oc1 (532 ± 13 mg L^{-1} GAE) and the highest in Oc5 (1 629 ± 29 mg L^{-1} GAE); the differences between orange juices were up to approximately threefold. Practically identical values were found for grapefruit juices made from concentrate

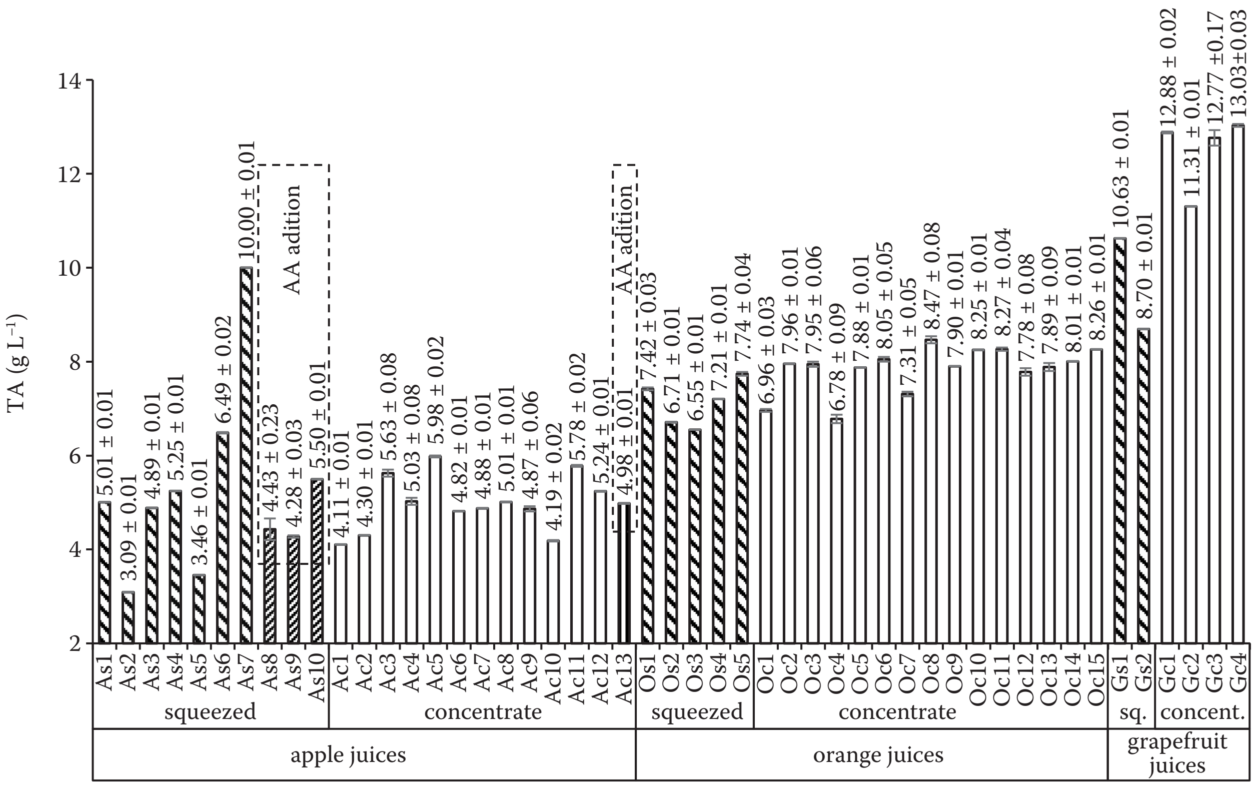

Figure 2. Titratable acids (TA) in 100% apple (A), orange (O) and grapefruit (G) juices

Pressed (s) (As8–As10) and concentrate (c) (Ac13) apple juices were fortified with vitamin C by the producers and are marked with different shading and marked with dashed rectangles; AA – ascorbic acid

(Gc 1 016 ± 164 mg L^{-1} GAE) and those that were directly pressed (Gs 1 092 ± 159 mg L^{-1} GAE), with the lowest polyphenol content in Gc1 (817 ± 4 mg L^{-1} GAE) and the highest in Gc4 (1 270 ± 21 mg L^{-1} GAE); the differences between grapefruit juices were at maximum up to 56%. Rekha et al. (2012) determined the content of total polyphenols in juices made from unripe and ripe citrus fruits. The polyphenol content ranged from 532 mg L^{-1} to 960 mg L^{-1}.

FN. FN values in mL of 0.1 M NaOH ranged from 2.8 mL (Ac11) to 25.5 mL (Os5) for the analysed juices. Both apple juices (Table 1) contained statistically significantly fewer free amino acids (As 4.81 mL and Ac 4.17 mL) than both grapefruit (Gs 15.93 mL and Gc 17.51 mL) and orange (Os 23.51 mL and Oc 20.24 mL) juices. For all three types of juices, no statistical differences between the production methods (from concentrate *vs.* directly pressed) were identified.

MA. MA content ranged from 3.90 g L^{-1} (As5) to 10.27 g L^{-1} (As7) for apple juices, from 0.21 g L^{-1} (Oc6) to 2.03 g L^{-1} (Os3) for orange juices and from 0.15 g L^{-1} (Gs2) to 0.31 g L^{-1} (Gc4) for grapefruit juices. Statistical differences (Table 1) were caused only by the fruit type (apples As 6.02 ± 1.93 g L^{-1}, Ac 6.87 ± 0.81 g L^{-1} *vs.* oranges Os 1.48 ± 0.49 g L^{-1}, Oc 0.99 ± 0.29 g L^{-1} and *vs.* grapefruit Gs 0.20 ± 0.07 g L^{-1}, Gc 0.26 ± 0.05 g L^{-1}), but not by production methods (directly pressed *vs.* from concentrate).

CA. The amount of CA ranged from 0.05 g L^{-1} (As2) to 0.22 g L^{-1} (Ac2) for apple juice, from 6.15 g L^{-1} (Oc5) to 12.00 g L^{-1} (Os2) for orange juice and from 12.10 g L^{-1} (Gs2) up to 16.50 g L^{-1} (Gs2) for grapefruit juice. Statistical differences were only based on the fruit type (Table 1), similarly like those for MA (apples As 0.12 ± ± 0.06 g L^{-1}, Ac 0.11 ± 0.06 g L^{-1} *vs.* oranges Os 8.95 ± ± 1.49 g L^{-1}, Oc 9.54 ± 1.67 g L^{-1} and *vs.* grapefruits Gs 15.59 ± 0.78 g L^{-1}, Gc 14.19 ± 1.83 g L^{-1}) but they were not caused by the production methods (from concentrate *vs.* directly pressed), analogously to MA.

VA. VA content ranged from 0.029 mg L^{-1} (Gs2) to 0.245 mg L^{-1} (Os4). There was a statistically significant difference (Table 1) only between directly pressed orange juice (Os 0.13 ± 0.06 mg L^{-1}) and grapefruit juice made from concentrate (Gc 0.05 ± 0.02 mg L^{-1}).

SS. SS values of the analysed juices ranged from 9.9 °Bx (Gc1) to 12.7 °Bx (Oc8). There were no statistically sig-

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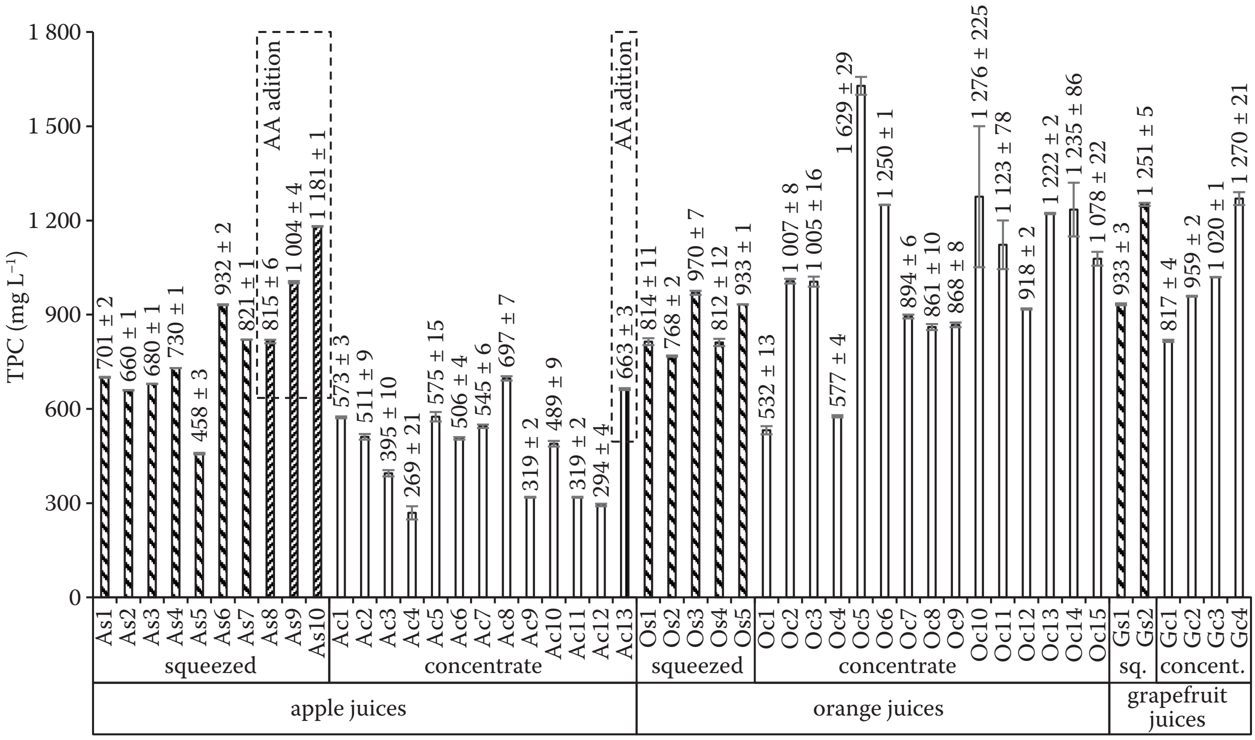

Figure 3. Total polyphenols content (TPC) in 100% apple (A), orange (O) and grapefruit (G) juices

Pressed (s) (As8–As10) and concentrate (c) (Ac13) apple juices were fortified with vitamin C by the producers and are marked with different shading and marked with dashed rectangles; AA – ascorbic acid

nificant differences in SS content between all juices (As, Ac, Os, Oc, Gs), except for grapefruit juice made from concentrate (Gc) (Table 1).

The density. The density of the juices ranged from 1.039 g mL^{-1} (Gc1) to 1.051 g mL^{-1} (Oc8). No statistical differences between the different types of juices (As, Ac, Os, Gs, and Gc) were observed. The densities of the juices were practically the same around the value of 1.045 g mL^{-1} (Table 1).

CONCLUSION

Forty-nine apple, orange, and grapefruit juices produced either from concentrate or directly pressed and available on the Czech market were analysed. According to the manufacturers, some of the apple juices contained added vitamin C, and in such juices, the AA content, instead of being around 50 mg L^{-1}, was comparable to orange and grapefruit juices with values ranging from 200 mg L^{-1} to 780 mg L^{-1}. The juices with added vitamin C did not have perceptibly higher TA contents than apple juices without any added vitamin C. The content of TA was practically the same as (or correlated with) the content of the dominant acid – about 5 g L^{-1} MA for apple juices and about 7 g L^{-1} CA for the orange and grapefruit juices; as might be expected based on taste, grapefruit juices were the most and apple juices the least acidic ones.

When comparing concentrate *vs.* directly pressed production technologies, all values are statistically insignificant except for the TA content of grapefruit juices, which was around 12 g L^{-1} for the concentrate juices, but only around 10 g L^{-1} for the directly pressed juices, and also except for the polyphenol content of the apple juices, where the polyphenol content of the apple juices from concentrate was about half that of the directly pressed juices (approximately 500 mg L^{-1}), which had similar values to the other types of juices (orange and grapefruit juices obtained by both methods).

For all juices, the density was around 1.04 g mL^{-1}, the VA content in hundredths of g L^{-1} and the SS around 11 °Bx.

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