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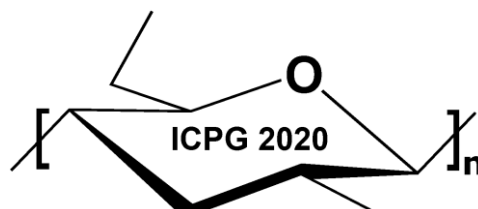
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Polysaccharides-Glycoscience**
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**Editors:
Radmila Řápková,
Czech Chemical Society
Novotného Lávká 5
Prague
Czech Republic**

**Markéta Bůžková, Prague
Jana Čopíková, Evžen Šárka,
UCT Prague
Department of Carbohydrates and Cereals
Technická 3
Prague
Czech Republic**

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Proceedings of the 16th International Conference on Polysaccharides-Glycoscience

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CARBOHYDRATE CONTENT OF “FLORINA” APPLES GROWN UNDER ORGANIC AND CONVENTIONAL FARMING SYSTEMS

**NADEZHDA PETKOVA^{a*}, MANOL OGNYANOV^b, SAMANTA KUZMANOVA^a, TATYANA BILEVA^c,
EKATERINA VALCHEVA^c, GALYA DOBREVSKA^d, NELI GROZEVA^c**

^a Department of Organic Chemistry and Inorganic Chemistry, University of Food Technologies, 26 Maritza Blvd., 4002, Plovdiv, ^b Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Laboratory of Biologically Active Substances, 139 Ruski Blvd., 4000 Plovdiv, ^c Department of Agroecology and Environmental protection, Agriculture University, 12 Mendeleev Blvd., Plovdiv, ^d Department of Fruit Growing, Agriculture University, Plovdiv, ^e Trakia University, Faculty of Agriculture, 6000 Stara Zagora, Bulgaria
petkovanadejda@abv.bg

Abstract

The aim of the current study was to evaluate the carbohydrate content of apple fruit cultivar “Florina” grown under organic and conventional farming systems. The total carbohydrate content was in the range from 67.8 to 63.6 g/100 g dry weight. Sucrose, fructose, glucose, and sorbitol were detected in all apple samples, as their content was the highest in the organically grown fruits. In general, organically grown apples contained the highest level of polysaccharides as pectin (4.6% g/100 g dry weight) and cellulose (4.6% g/100 g dry weight). In conclusion, the organically grown apples were evaluated as the sweetest ones with sweetness index (SI) 69 and with the highest fiber content, due to the high cellulose and pectin content, respectively.

Introduction

Apples are one of the most important and widely distributed fruit crop in temperate climate zones, and their production is increasing. For 2018 the apple production in Bulgaria occupies 22% of the total fruit production as the increase with 12% (50 298 tone) was found in comparison with the apple production for the last year¹. The main apple cultivars in Bulgaria include “Golden Delicious” (19.62%), “Red Delicious” (9.29%), “Granny Smith” (8.51%), “Florina” (7.68%), “Melrose” (6.26%)². Each of the apple cultivars has its own significant chemical composition. However, differences in it may occur depending on the maturity, environmental factors, horticultural practices applied in an orchard and storage conditions³⁻⁵.

“Florina” apples also called “Querina” is a French cultivar of domesticated apple, that has combined traits of the “Jonathan”, “Golden Delicious” and “Rome Beauty” apples obtained in Angers, France in 1977⁶. Reaches full ripeness around 25th – 30th September and it produced the highest mean yields – 11.7–14.7 t/ha (ref.⁷). The “Florina” apples are resistant to the economically most significant disease on the apples – scab. The main colour of the fruit is yellow-green, almost entirely covered by red to dark red stripes. Its flesh is creamy-coloured, mild, medium firm, juicy, a blend of sweet and tart, aromatic and of good quality. In cool storage the fruit can be kept for six months⁸. “Florina” is among the most desired apple varieties for fresh consumption⁶⁻⁸.

The carbohydrate composition and organic acid profile of fruit is an important component of chemical composition and provides valuable information about the authenticity of fruit products^{3,4} and influence on the sensory properties and nutritional value of fruits. It is known that the sugar composition in apples consists of sucrose, glucose, fructose and sorbitol. However, the quality and sweetness of apple fruits strongly depend on the proportion of these sugars^{4,9,10}. Fructose is the main sugar in apple fruits, which values can reach to 78% of the total sugar content¹⁰, while sorbitol represents less than 10% of the total sugar⁹. It was reported that soluble sugars contribute to a range of fruit quality traits such as flavour, texture and healthy properties^{3,4,9}. Taste is related to water-soluble and non-volatile compounds, while sweetness is mostly attributable to mono- and disaccharides. The sugar/acid ratio is responsible for the taste and flavour of apples^{3,10}. However, the evaluation of carbohydrate composition and total acidity in “Florina” apples grown under different conditions is still missing. Until now no detailed analysis of the carbohydrate composition of “Florina” apple and its sweetness was found. Therefore the aim of the current study was to evaluate the carbohydrate content of apple fruit cultivar “Florina” grown under organic and conventional farming systems.

Material and methods

Material

All the solvents and reagents were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Fillab (Plovdiv, Bulgaria). The reagents were used directly without further pretreatment.

Plant growing

The research activities were realized on the training and experimental fields, spreading round the city of Plovdiv and the village of Brestnik. The study was conducted in the orchards with “Florina” cultivar – grafted on MM106 rootstock at the Agroecological Centre of the Agricultural University of Plovdiv (42.133845 N, 24.807315 E) and Brestnik village. Four groups of four trees per orchard were randomly chosen as sample trees. The apple orchards were cultivated under organic and conventional agricultural practices. Additionally, turf-mulching system were applied in the sward apple orchard.

Conventional orchard: In May and during the five years orchard was treated with the fungicides Tiram 80 VG – 0.3%, Follicur 250 EV – 0.04% (broad spectrum system), Delan 700 VDG – 0.05%, Score 250 EC – 0.02%, Horus 50 EC – 0.03%, Shavit F 72 VDG – 0.2% and Bayfidan 250 EC (systemic) – 0.015%. Against cross moth, lice and mites apple plants were treated again a different number of times in different years with Nurelle D – 0.04%, Fury 10 EC – 0.125% (contact synthetic pyrethroid of a new generation) and Coragen 20 SC – 16 mL/ha.

Organic orchard: Shampion VP – 0.15% (end of November) and Funguran ON – 0.3% (contact broad-spectrum, containing 77% Cu-hydroxide) combined with Colloidal sulfur – 1:400. Trifolio S Forte 0.3% (50% vegetable oil + 50% emulsifier) and

Acarzin 3% (85% mineral oil + 15% emulsifier) were used against the hibernating forms of aphids, apple fruit worm. Post-blossoming treatments in May was performed against apple diseases and pests mainly target *Venturia inaequalis*, *Podosphaera leucotricha*, aphids and apple fruit worm with uses of the antifungal agent Kuore 200g/dka (contains 10% Cu and 1.1% Zn, Colloidal sulfur – 1:400, Nimazal T/C – 200 mL/dka (a bio insecticide) + Trifolio S Forte – 0.3%. Pheromone trapping was used to control apple fruit worm.

General methods

Moisture content (%) of the apple was determined after drying at 105 °C until constant weight (AOAC, 2007)¹¹. Ash content (%) was determined by igniting the sample in a muffle furnace (MLW, Germany) at 550°C for 4 h (AOAC, 2007)¹¹. pH was measured using a pH meter 7110 WTW (Weilheim, Germany). Titratable acidity (TA) was measured by titration with 0.1M NaOH to the pH value of 8.1 and the result was expressed as g of malic acid equivalent per 100 g fresh weight (fw) ISO standard¹².

Total soluble carbohydrates

The total soluble carbohydrate content was evaluated by phenol-sulphuric acid method. In brief, 100 µL properly diluted hydrolyzed extract was mixed with 1 mL 5% phenol and 5 mL concentrated H₂SO₄¹³. The samples were placed in a water bath at 30° C for 20 min and then the absorbance was measured at 490 nm against blank with d. H₂O. The amount of carbohydrates was determined from the calibration curve constructed with glucose. The results were calculated as a percent (%) of dry weight (dw).

Total uronic acid content (pectin)

The uronic acid content of apple fruits was determined according to the method of Ahmed and Labavitch¹⁷. Before analysis, the sample was solubilized with 72% (w/w) H₂SO₄ for 1 h at 30 °C, followed by hydrolysis step with 1M H₂SO₄ for 3 h at 100 °C. An aliquot of hydrolyzate was used for analysis by *m*-hydroxydiphenyl assay using galacturonic acid as a standard¹⁸.

Crude cellulose

The finely ground sample (0.5 to 1 g) was placed in a round bottom flask of 250 mL and 16,5 cm³ acid mixture containing 80% acetic acid and nitric acid were added (450 cm³ 80% acetic acid was mixed with 45 cm³ concentrated nitric acid). The flask with sample was heated at boiling temperature for 30 min with periodically shaking. Then the sample was filtered through previously tarred sintered glass (G2) filter. The sample was washed with the hot acid mixture, then with hot water and 95% ethanol to remove the residual acids. After that 10 cm³ diethyl ether was added to the residue on the sintered glass filter and the washing process were repeated again with hot water, 95% ethanol and diethyl ether. At the end the sintered glass with the residual sample were dried to the constant weight in an oven at 105 °C, cool down in a desiccator and then weighted¹⁹.

HPLC analysis of sugars and polyols

Preparation of apple water extracts was performed on a solid to liquid ratio 1:5 (w/v) with distilled water in an ultrasonic bath (VWR, Malaysia) with frequency 45 kHz and 30W power at 45 °C in triplicate¹⁴. The samples were filtered. The content of sugars and sorbitol were evaluated by HPLC-RID method. Analysis was performed on HPLC Shimadzu, coupled with LC-20 AD pump, and a refractive index detector (RID) Shimadzu RID-10A. The separation was done on a Shodex® Sugar SP0810 (300 mm×8.0 mm i.d.) column with Pb²⁺ and a guard column Shodex SP-G (5 µm, 6 mm×50 mm) (Shodex Co., Tokyo, Japan) operating at 85°C, mobile phase d. H₂O with flow rate of 0.5 mL/min and the injection volume 20 µL (ref.¹⁵).

Sweetness

Sweetness index (SI) and total sweetness index (TSI) were calculated based on the results of HPLC analysis of the individual sugars for determination of the fruit sweetness perception¹⁶. Sweetness Index – SI was calculated, assuming that fructose and sucrose are 2.30 and 1.35 times sweeter than glucose, respectively: $SI = (1.00 \times [\text{glucose}]) + (2.30 \times [\text{fructose}]) + (1.35 \times [\text{sucrose}])$. Total sweetness index – TSI is expressed with the contribution of each sugar, estimated relative to sucrose: $TSI = (1.00 \times [\text{sucrose}]) + (0.76 \times [\text{glucose}]) + (1.50 \times [\text{fructose}])$.

Statistical analysis

All analyses were performed in triplicate (n = 3). The data were presented as a mean values±standard deviation (SD). Statistical analysis was performed using MS Excel 2010.

Results and discussion

The obtained characteristics of “Florina” apple fruits grown under different conditions were summarized in Table I. The moisture content of “Florina” apple fruits was in the range of 79.90 to 81.98% fresh weight, while the dry matter was between 18.02 to 20.10%. Our results were close to other apple variety^{2,3,10} and consistent with “Florina” apples grown in Romania²⁰. However, the organically grown “Florina” apple in Bulgaria demonstrated higher dry matter in a comparison to the same organically grown apple cultivar and integrated systems in the Czech Republic²¹. Moreover, the displayed tendency for apples from organic farming to have a higher amount of dry matter than the apples from integrated system²¹ was also observed in our study. Titratable acidity varied from 0.24 to 0.54, as was the highest in organic sward orchard fruits 0.54%, while pH was in all samples around 4.0. Close to our observed pH values were reported for Romanian “Florina” apple cultivars pH 4.2 (ref.⁸). The pH of apple juice used for cider production must not be greater than 3.6, therefore, “Florina” apple fruits is recommended to be consumed fresh not as a cider production. Low acidity determines a good quality for human consumption²². In our study titratable acidity did not differ between the organic and conventional growing conditions. Roussos and Gasparatos²³ reported the same tendency. They did not find any differences in the titratable acidity between the two production practices.

Table I

The characterization of “Florina” apple fruits grown under different conditions

Characteristics	Conventional orchard	Conventional sward orchard	Organic orchard	Organic sward orchard
Moisture content, %	81.40±2.50	81.98±0.58	80.90±1.45	79.90±1.20
Dry matter, %	18.60±2.50	18.02±0.58	19.10±1.45	20.10±1.20
pH	4.00±0.11	3.99±0.03	4.05±0.08	3.97±0.02
Titration acidity, %	0.24±0.02	0.43±0.13	0.48±0.18	0.54±0.23
Total carbohydrates, g/100 g dw	67.79±2.20	63.58±2.00	67.01±1.50	64.64±1.20
Total uronic acids (pectin), g/100 g dw	3.84±0.10	3.70±0.10	4.59±0.20	4.34±0.11
Cellulose, g/100 g dw	6.85±0.10	4.34±0.15	4.63±0.07	4.48±0.05
Total sugars, g/100 g dw	35.55±0.60	19.62±1.45	40.12±2.20	19.69±1.25
Glucose, g/100 g dw	10.09±0.21	4.92±0.79	7.97±0.24	4.86±0.22
Fructose, g/100 g dw	16.69±0.63	9.15±1.25	18.79±4.08	8.95±1.28
Sucrose, g/100 g dw	7.96±0.11	5.55±0.38	13.36±0.50	5.88±0.21
Sorbitol, g/100 g dw	2.79±0.46	3.37±0.32	4.22±0.24	2.45±0.21
Glucose/Fructose	0.65	0.54	0.42	0.54
Sucrose/Glucose	0.73	1.13	1.68	1.21

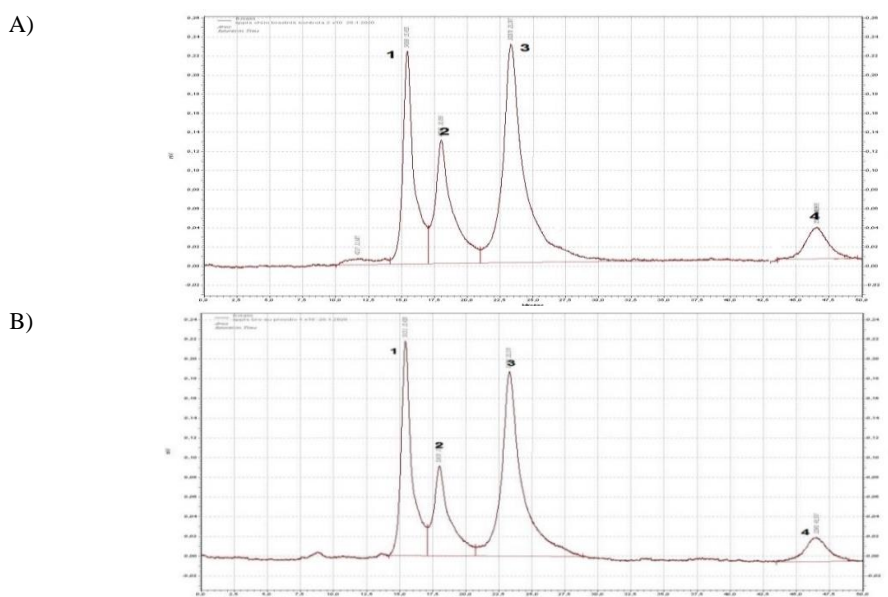


Fig. 1. HPLC-RID chromatogram of the water extract from apple “Florina” collected from A) conventional sward orchard and B) organic orchard, where 1. sucrose, 2. glucose, 3. fructose and 4. sorbitol

The carbohydrate content varied from 63.58 to 67.79% dw, as the highest values were detected in conventional and organic orchard. In these samples more than 60% were due to the soluble sugars and approximately 15% were due to dietary fibers represented as pectin and cellulose. These fibers are associated with healthy nutrition and are responsible for the beneficial effects of apple consumption. In the current study the pectin content slightly dominated in all organically grown apple fruits, as its values reached 4.59%, while cellulose content dominated in conventional “Florina” apples – 6.85%. In our study reported values for cellulose content in apple were higher than earlier reported values around 0.9 to 1% (ref.²⁴). Moreover, a large-scale experimental analysis showed that 196.0 g of pectin and 243.9 g of cellulose (90.4% purity) was collectively prepared from 1000 g of apple pomace²⁵. The pectin and pigments compose only 20% of apple pomace weight, and therefore, more mass was usually left as solid waste after the extraction processes²⁶.

In all tested samples the presence of sucrose, glucose, fructose and sorbitol were detected (Table I and Fig. 1). Glucose content varied between 4.82 to 10.09%, as the highest values were found in the apples from conventional orchards. Sucrose content was between 5.55 to 13.36 %, while sorbitol were between 2.45 to 4.44%. The main part of the total sugar content was due to fructose, as its content was the highest in the organic orchard – 18.79% dw. The highest concentration of fructose in apple fruit had the highest impact on their sweetness. In general, the content of sucrose, fructose and sorbitol in apple fruits from organic orchards were the highest in comparison with conventional growing conditions and organic sward. Similar observation for domination of fructose was observed for other apple varieties, especially “Golden Delicious”²⁷. Contrary to some reports, apples grown organically contained higher levels of total sugars, as well as a higher content of glucose, fructose and sucrose in comparison with conventionally produced apples.

The ratio between glucose and fructose, as well as sucrose to glucose were also defined. Differences in glucose and fructose contents are reflected in the glucose/fructose ratio that was in the range from 0.42 to 0.65 and it were comparable with values reported by Begić-Akagić⁴ for some apple varieties from Bosnia and Herzegovina- “Kanjiska”, “Paradija”, “Red Delicious” and “Granny Smith”. The ratio between sucrose and glucose were the highest in organically grown apples – from 1.21 to 1.68. The content of sugars and organic acids depend on the plant genotype and is also influenced by environmental

factors such as the weather conditions, cultivars, position, and exposition of the fruits in the crown as well as horticultural practice undertaken in an orchard^{3,10, 22}.

Table II
Sweetness and sourness of apple “Florina”

Apple sample	SI	TSI	TS/TA
Conventional orchard	60.03	41.28	148.13
Conventional sward orchard	33.46	23.01	45.63
Organic orchard	69.22	47.60	83.58
Organic sward orchard	33.38	22.99	36.46

The values of SI and TSI were higher in apples from the organic orchard, followed by apples from conventional orchard. However, apples from sward orchard demonstrated twice lower SI and TSI values. Therefore, this type of growing conditions decreased the sweetness of fruits. The ratio between the analyzed total sugars and organic acids is an indicator of the internal quality of fruits as it reveals their flavor. It is also an indicator of commercial and sensory ripeness. The fruits that are characterized by sugar/organic acid ratio in the range from 3 to 9, are described as having a sweet-sour or sour-sweet taste²⁸. In our case, total sugars to total acid (TS/TA) ratio varied between 148 to 36 (Table II) that showed the sweetness of fruits. According to reported researches apple cultivars with sugar/acid ratios below 20 are acidic and are suitable for processing and cider production, while cultivars with sugar/acid ratios higher than this value are sweet, and good for direct consumption^{3,4}. Moreover, fertilization also changed the taste of apple fruit, which was assessed on the basis of the sugar/acid ratio (TS/TA). Our data for “Florina” apple were consistent with the sugar to acid ratio of the tested cultivars from Bosna and Herigovina that were in the range from 5.36 (‘GS’) to 189.90 (ref.⁴). In comparison apple cultivar Idared grown organically demonstrated TS/TA ratio between 17 and 28^[3]. Therefore, the apple growing in sward orchard strongly affected TS/TA ratio.

Conclusion

To the best of our knowledge this is the first detailed report providing information about the carbohydrate content in “Florina” cultivars grown under different farming system conditions. In addition, important indicators characterizing the organoleptic properties of the apple fruit were evaluated. From the obtained results it can be concluded that “Florina” apples grown conventionally and organically are sweet, rich in dietary fibers and suitable for fresh consumption. These data are an appropriate basis for the choice of cultivation techniques for individual varieties of fruit, as well as for their subsequent food processing.

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