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Investigation of the nutritional and health values of apple land varieties

Keywords: old apple varieties, Malus × domestica, fruit quality, functional food

1. SUMMARY

In our research, we sought to answer the question whether the health-protecting values attributed to land varieties in folk medicine can be substantiated by laboratory analyses. In our experiments, nutritional (sugar and acid components) and health values (total antioxidant capacity and total polyphenol content) of eight apple land varieties were examined. Half of the land varieties investigated reached the fruit quality of the control 'Golden Delicious'. Due to their very favorable glucose-to-fructose ratio, the consumption of 'Bordás alma', 'Nyári édesalma', 'Jóalma' and 'Kapitány alma' is more favorable in the diet of diabetic patients. The antioxidant capacity of 'Pirosló bőralma' and 'Jóalma', as well as the polyphenol content of 'Piros pogácsa alma', 'Kapitány alma' and 'Vasalma' are outstanding, two to three times higher than those of the control varieties. Land varieties with a favorable sugar composition and high antioxidant and polyphenol content can be used as functional foods.

2. Introduction

Tens of thousands of apple varieties are known worldwide, but the variety use of industrial fruit production is predominantly limited to 5 to 10 varieties and their mutants, and variety use varies in part from country to country. Our old fruit varieties and land varieties cannot hold their own in industrial scale production, but they can play a significant role in local markets and fresh consumption.

According to Paragraph 1 of VM decree 27/2012 (III. 24.) on the state recognition of fruit land varieties and the conditions for the production and marketing of their propagating materials a land variety is "a fruit variety naturally adapted to regional, environmental and local ecological conditions and endangered by genetic erosion" [1]. The local variety is a variety grown in certain areas for subsistence or sale at nearby markets. By the 1800s, a special, unique variety structure evolved in Hungary. Landscape frameworks and characteristics had a strong determining effect on the development of land varieties. The exact way they originated is obscured by time, and today they are known as characteristic varieties of certain regions [2, 3].

Old or land varieties that can still be found in some places in old orchards are of significant genetic and cultural value, which are of key importance for the conservation of biodiversity. However, the condition of the old scattered orchards has greatly deteriorated since the old owners stopped cultivating them. The condition of unpruned, unmowed orchards is getting worse year by year [4]. *In situ* conservation of old varieties is difficult to achieve due to environmental and anthropogenic factors. *Ex situ* conservation is possible in newly established land orchards, educational gardens or plant gene banks. There are many state and private fruit gene banks in Hungary [5].

In his book "*Gyümölcsöző sokféleség*" (Fruitful Diversity) **[6]**, Surányi evaluates the advantages and disadvantages of old varieties and local varieties in terms of their scientific, economic and socio-cultural impacts. Overall, he finds that the use of historical and land varieties increases biodiversity, contributes to the success of landscape and nature conservation, provides healthier food and a better livelihood for the population of underdeveloped areas.

In terms of morphological properties, a relatively large number of textbooks are available **[5, 6, 7, 8, 9, 10,**

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11, 12, 13], although these pomological works were mainly created in the 19th century. Given our common historical past, the description of certain Hungarian varieties can be found in pomological works written in some neighboring countries **[14, 15]**.

There are only a few articles and studies on old Hungarian varieties, and they mainly report the results of morphological, phenological or resistance studies carried out in gene bank collections or scattered orchards **[2, 16, 17, 18, 19, 20, 21, 22, 23]**.

Apples are a popular fruit that can be consumed all year round, an integral part of a healthy diet. Apples have a water content of 90% and a carbohydrate content of 9 to 14%, hence their energy content is low, 31 kcal (i.e., 130 kJ), so those on a weight loss program can consume them in unlimited amounts. Their carbohydrate composition is very favorable, most of it is fructose (60%) [24, 25]. Due to the favorable glucose-tofructose ratio, regular consumption of apples stabilizes blood sugar levels, they can be consumed by diabetic patients as well in controlled amounts. They also have excellent dietary and health effects due to their high fiber and pectin contents (0.8% and 1.3%). Of organic acids, malic acid has the highest proportion, and citric acid, succinic acid, phosphoric acid and chlorogenic acid also occur. In terms of minerals, potassium, calcium, magnesium, phosphorus and trace elements are present in significant amounts [25]. Due to the high antioxidant and polyphenol content of the apple fruit, it plays a significant role in the protection against damage caused by oxidative stress, in the elimination of free radicals and in increasing the effectiveness of the antioxidant protection system [26].

Many of our land varieties have unique nutritional values and special areas of use, which has allowed these varieties to survive even today. Due to their nutritional and health protection values (e.g., outstanding polyphenol content), they can also be used as functional foods **[27, 28, 29, 30, 31, 32, 33]**.

Our study included apple land varieties, mainly from Western Hungary, which may be outstanding as functional foods due to their role in folk medicine. The water-soluble dry matter content, sugar and acid components, total polyphenol content and antioxidant capacity of the fruits were investigated.

3. Materials and methods

3.1. Varieties examined

The fruit samples (except for the control 'Golden Delicious') came from the Pórszombat gene bank of Gyula Kovács, where on sampling rootstocks are traditionally cultivated. The varieties examined are not found on the National List of Varieties, most of the varieties can be considered as typical Göcsej land varieties.

The fruit quality of eight apple varieties ('Jóalma', 'Rétesalma', 'Bordás alma', 'Pirosló bőralma', 'Nyári édesalma', 'Piros pogácsa alma', 'Vasalma', 'Kapitány alma') (*Figure 1*) was compared to the fruit of the control 'Golden Delicious' and 'Gala' varieties. Two samples of 'Golden Delicious' were examined: one from the Pórszombat gene bank, from traditional cultivation, and a commercially available one, presumably from industrial production.



Figure 1. Apple varieties included in the experiment (from left to right: 'Jóalma', 'Rétesalma', 'Bordás alma', 'Pirosló bőralma', 'Nyári édesalma', 'Piros pogácsa alma', 'Vasalma', 'Kapitány alma', 'Golden Delicious' (Pórszombat))

Our study included land varieties from Western Hungary with which certain folk medicinal effects are associated. For example, 'Jóalma' were taken to mothers-to-be at birth, because it was thought that if she consumed them, the child would not be colicky. 'Piros pogácsa alma' can be consumed by diabetic patients as well. The characterization and usability of the fruits can be found on the website of the "*Tündérkert*" (Fairy Garden) operated by Gyula Kovács **[34]**.

3.2. Examined fruit nutritional and health values

The measurements were performed at the Faculty of Horticulture and Rural Development of John von Neumann University and the Department of Food Science of the Faculty of Agricultural and Food Sciences of Széchenyi István University. The nutritional (water-soluble dry matter content (refraction), sugar and acid components) and health values (polyphenols, antioxidants) of the fruits of the varieties were investigated.

Measurement of water-soluble dry matter content

The fruits were harvested at consumption maturity (**Table 1**), and rapid laboratory tests (refraction) were carried out within 1 to 2 days after sample collection. 10 to 20 pieces of fruit per variety were available for testing. The water-soluble dry matter content (refraction) was determined from a homogeneous, filtered fruit juice with a hand-held refractometer, and the results were expressed in Brix%, i.e., g/100 g. With the sampling drill supplied with the device, samples were taken from several points from each fruit, so we were able to perform 4 to 6 measurements per fruit. The data obtained during the study were averaged by variety.

3.2.1. Determination of organic acid and sugar components

Fruits were frozen after harvesting. During sample preparation, the flesh of the fruit samples was minced and blended immediately before the measurement. For the measurements, 1 g of the samples was weighed into an Erlenmeyer flask, 50 ml of distilled water was added, then it was stirred on a magnetic stirrer for 60 minutes and, finally, centrifuged (30 minutes, 5,500 g). 1.5 ml was transferred into an Eppendorf tube and, after centrifugation (24,500 g, 20 minutes), 1 ml of the supernatant was filtered into a 1.8 ml vial. The samples thus prepared were injected into the HPLC instrument.

Organic acids were determined by ion exclusion liquid chromatography. The following standards were used: malic acid (95% pure), succinic acid (99% pure), citric acid (98% pure). To prepare standard stock solutions, standards were dissolved in 0.1% H_2SO_4 solution to the desired concentration (10 mg/ mL). The JASCO HPLC instrument consisted of the following units: pump (PU-980), autosampler (AS-950-10), degasser (VWR Model 2004), detector (UV-975), column thermostat (Jones Chromatography Model 7955), column (Bio-Rad Aminex HPX-87H). Measurement conditions were as follows: column temperature: 35 °C; flow rate: 0.6 ml/min; detection wavelength: 210 nm; eluent: 0.1% H_2SO_4 (isocratic).

Separation of the sugars was performed on an ion exclusion column by reverse phase liquid chromatography. The following standards were used: fructose (99% pure), glucose (99.5% pure), sucrose (99.5% pure). To prepare standard stock solutions, standards were dissolved in 100% H_2SO_4 solution to the desired concentration (10 mg/mL).

| Variety | Harvest date |
|--|--------------------|
| Jóalma (Good apples) | July 16, 2019 |
| Rétesalma (Strudel apples) | September 16, 2019 |
| Bordás alma (Ribbed apples) | September 16, 2019 |
| Pirosló bőralma (Reddish skin apples) | September 16, 2019 |
| Nyári édesalma (Summer sweet apples) | September 16, 2019 |
| Piros pogácsa alma (Red cake apples) | November 04, 2019 |
| Vasalma (Iron apples) | November 04, 2019 |
| Kapitány alma (Captain apples) | November 04, 2019 |
| Golden Delicious (Golden Delicious) | November 04, 2019 |
| Golden Delicious (kontroll) (Golden Delicious (control)) | November 08, 2019* |
| *date of purchase, commercially available sample | |

Table 1. Sample collection dates (Pórszombat, 2019)

*date of purchase, commercially available sample

The JASCO HPLC system used consisted of the following units: pump (PU-980); autosampler (AS-2055); degasser (DG-1580-53); detector (Refractive index detector, Merck RI 71); column (Supelcogel H, Sigma-Aldrich). Measurement conditions were as follows: column temperature: room temperature; flow rate: 0.5 ml/min; eluent: 100% H_2O (isocratic).

Malic acid, citric acid, succinic acid, fructose, glucose and sucrose concentrations of the apple samples were determined using the following formula:

$$C = \frac{(A-b)*V}{a*m}$$
(1)

where:

- c: concentration of the given component
- A: peak area of the given component
- A_{ad}: peak area of the given additioned component
- b: intercept of the analytical measurement curve
- a: sensitivity of the measurement system (slope of the analytical measurement curve)
- V: dilution volume of the sample solution (100 mL)
- m: mass of the weighed fruit sample.

3.2.2. Determination of the total antioxidant capacity (FRAP) and the total polyphenol content

Fruits were frozen after harvesting. During sample preparation, the flesh of the fruit samples was minced and blended immediately before the measurement. 20 g of the minced sample was stirred on a magnetic stirrer for 1 hour with an extraction mixture containing methanol (70 ml), 37% hydrochloric acid (0.1 ml) and deionized water (29 ml), then it was centrifuged for 20 minutes at 10 °C and 5,500 g. The extract thus prepared was used to determine the antioxidant and polyphenol content.

Total antioxidant capacity was determined by the modified FRAP method of Benzie and Strain **[35]**. To 100 μ I of the extract were added 3 mI of FRAP solution and 100 μ I of high purity water. This was measured against an extract not containing fruit, at 593 nm, with a spectrophotometer.

For the determination of the total polyphenol content, to 100 μ l of the extract 1.5 ml of high purity water was added, followed by the addition of the reagents. First, 2.5 ml of Folin reagent, then 2 ml of Na₂CO₃. Absorbance was measured after 90 minutes at 750 nm, against an extract not containing fruit, with a spectrophotometer.

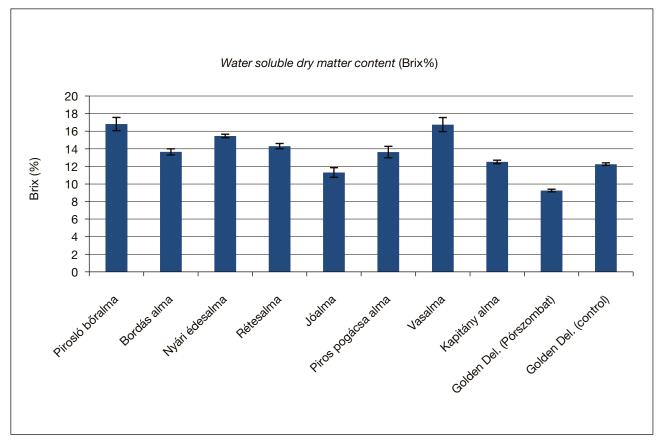


Figure 2. Water-soluble dry matter content of apple land varieties (refraction) (Pórszombat, 2019)

The same procedures were followed for the ascorbic acid solutions of 40, 60, 80, 100, 150, 200, 250 and 500 mg/l concentrations prepared for the calibration of the spectrophotometer for the determination of antioxidant capacity and the gallic acid solutions of the same concentrations prepared for the determination of polyphenol content. The total antioxidant and total polyphenol content of the apple samples were determined from the absorbance values measured for the apple sample solutions using the second order equation of the analytical curve fitted to the absorbance values measured for the calibration solutions and the corresponding concentration values using the nonlinear least squares method.

The total antioxidant and total polyphenol concentration of the apple fruits were determined using the following formula:

$$C = \frac{100*A}{a*m}$$
 (2)

where:

- c: concentration of the given component
- A: absorbance of the given component
- V: dilution volume of the sample solution (100 mL)
- m: weight of strawberries measured
- a: slope of the analytical measurement curve.

4. Results and discussion

4.1. Water-soluble dry matter content

The water-soluble dry matter content (11-17 Brix%) of the land varieties examined reached or exceeded that of the 'Golden Delicious' control variety (Figure 2). The Brix% of the 'Golden Delicious' from the Pórszombat orchard was lower than that from the industrial orchard. In our previous work [33], a water-soluble dry matter content of 12 Brix% was also measured for the 'Golden Delicious' variety. Varieties with a Brix% over 12 are suitable for the production of concentrate [36]. The land varieties examined, with the exception of 'Jóalma', are suitable for processing if their acid content is around 7 g/l. Previous Hungarian studies [28, 29, 30, 31, 32] also measured medium-high water-soluble dry matter contents (above 12 Brix%) in most old Hungarian apple varieties.

4.2. Sugar components

The measured sugar components (glucose, fructose, sucrose) account for 64 to 96% of the water-soluble dry matter content (Brix%). The sugar content of the varieties (glucose+fructose+sucrose) was 82-142 mg/g (average: 111 mg/g). The amounts of sugars were as follows (*Figure 3*): fructose: 50.4-72.6 mg/g (average: 63 mg/g), sucrose: 27.6-54.2 mg/g (average: 37,5 mg/g), glucose: 0.8-22.8 mg/g (average: 10.6 mg/g). When analyzing the sugar components, it is

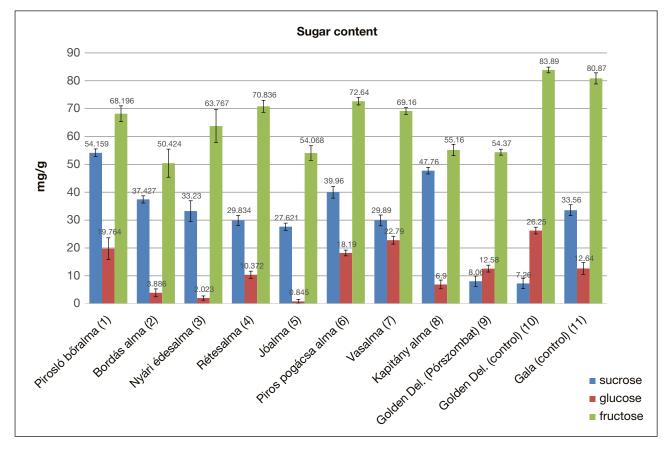


Figure 3. Sugar components of apple land varieties (Pórszombat, 2019)

clear that fructose (48-72%) is predominant in apple varieties, which is in agreement with the average value of 60% reported in textbooks (e.g., **[25]**). In land varieties, the proportion of sucrose was 27-41% and the proportion of glucose was 1-22%.

In addition to reducing the glucose intake in the diet of diabetic patients, the consumption of fructose and sucrose is more desirable. Fructose, as the sweetest sugar, imparts a sweet taste even at lower concentrations, but at the same time increases blood sugar levels more slowly due to its enzymatic conversion to glucose. Similarly, the disaccharide sucrose has a much smaller blood sugar raising effect. The glucose-to-fructose ratio of the fruits of the studied varieties was 0.02-0.33. Due to their very favorable glucose-to-fructose ratio (below 0.15), low glucose content (below 7 mg/g) and higher sucrose content, the consumption of 'Bordás alma', 'Nyári édesalma', 'Jóalma' and 'Kapitány alma' is more beneficial in the diet of diabetic patients. According to folk medicine, the 'Red cake apple' variety can also be consumed by diabetic patients. This was not clearly supported by our studies: its glucose-tofructose ratio is medium (0.25).

4.3. Organic acid components

In the apple fruit, malic acid is predominant (80-95%), but it also contains citric acid, succinic acid, and smaller amounts of phosphoric acid and chlorogenic acid **[25]**. The total acid content (malic acid + citric

acid + succinic acid) of the varieties examined was 10.2-18.7 mg/ml (average: 13.8 mg/ml). The fruits of 'Pirosló bőralma' (18.7 mg/ml) and 'Kapitány alma' (16.9 mg/ml) had exceedingly high acid contents. Despite its high acid content, 'Pirosló bőralma' has sweet and sour fruits because its sugar content is also high (142 mg/g).

The amounts of acids were as follows (*Figure 4*): malic acid: 6-12 mg/ml (average: 10.4 mg/ml), citric acid: 0-7.1 mg/ml (average: 2.7 mg/ml), succinic acid: 0.4-1.4 mg/ml (average: 0.7 mg/ml). Malic acid was dominant in the samples. The distribution of the organic acids in the varieties: malic acid (54-95%), citric acid: 0-44.3%, succinic acid: 2.1-10.3%.

4.4. Total antioxidant capacity (FRAP) and total polyphenol content

The total antioxidant capacity (FRAP) and total polyphenol content of the land varieties are shown in *Figure 5*. The total antioxidant capacity of the varieties studied was 2.14-4.22 mg AS/g (average: 3.46 mg AS/g). The antioxidant capacities of 'Pirosló bőralma', 'Jóalma', 'Piros pogácsa alma', 'Vasalma' and 'Kapitány alma' were twice as high as that of the 'Golden Delicious' control varieties was 0.24-2.75 mg GAE/g (average: 1.19 mg GAE/g). The polyphenol content of 'Piros pogácsa alma' and 'Kapitány alma' was twice as high, the polyphenol content of 'Vasalma' and 'Kapitány alma' was three times as high as that

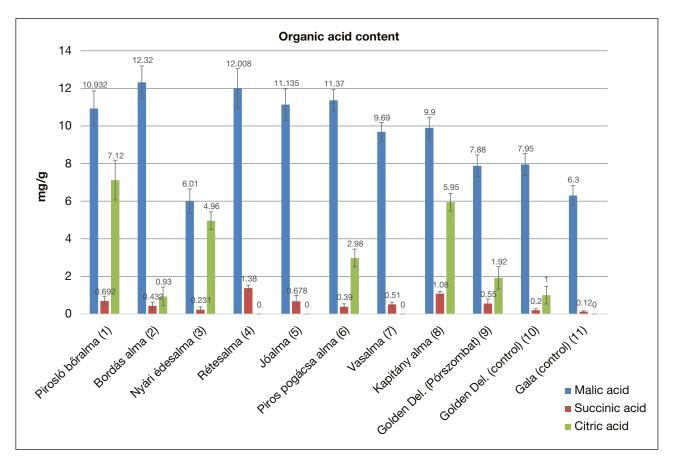


Figure 4. Acid components of apple land varieties (Pórszombat, 2019)

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of the 'Golden Delicious' and 'Gala' control varieties. In contrast, the other land varieties have much lower polyphenol contents. Other researchers have found similarly high antioxidant and polyphenol contents in the case of many old Hungarian apple varieties **[28, 32, 33]**.

5. Conclusions

In our research, we sought to answer the question whether the health-protecting values attributed to land varieties in folk medicine can be substantiated by laboratory analyses. The water-soluble dry matter content (Brix%) of land varieties from scattered orchards was slightly higher than that of the control varieties from industrial fruit production. Due to their very favorable glucose-to-fructose ratios, the consumption of 'Bordás alma', 'Nyári édesalma', 'Jóalma' and 'Kapitány alma' is preferred in the diet of diabetic patients. According to folk medicine, the 'Red cake apple' variety can also be consumed by diabetic patients. In our study, its glucose-to-fructose ratio was moderate, which is still adequate, but it is advisable to give preference to the above-mentioned varieties. The average distribution of organic acids in the land varieties shows a similar result as in the control: malic acid 77%, citric acid: 18%, succinic acid: 5%. The antioxidant capacity of 'Pirosló bőralma' and 'Jóalma', as well as the polyphenol content of 'Piros pogácsa alma', 'Kapitány alma' and 'Vasalma' are outstanding, two to three times higher than those of the control varieties. 'Jóalma' were taken to mothers-to-be at birth, because it was thought that if she consumed them, the child would not be colicky. This can be explained by the favorable glucose-to-fructose ratio and perhaps the high content of malic acid and polyphenols. Land varieties with a favorable sugar composition and high antioxidant and polyphenol content can be used as functional foods.

6. Acknowledgment

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In this paper, the authors report on the results of the examination of Hungarian apple varieties. Therefore, the Hungarian variety names are used in the text, but in **Table 1**. we also give their raw translation in English (A ed.).

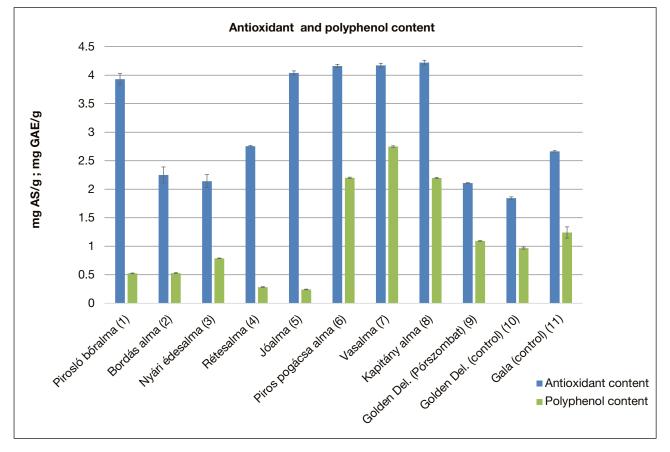


Figure 5. Total antioxidant capacity (FRAP) and total polyphenol content (Pórszombat, 2019)

7. Literature

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