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Mateusz Aninowski , [Katarzyna Mietlińska](#) , [Joanna Leszczyńska](#) * , [Radosław Bonikowski](#) , [Kamil Szyczak](#) *

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Article

Antioxidants and Allergens: Suitability of Selected Apple Varieties for Allergy Sufferers and Diabetics

Mateusz Aninowski ¹, Katarzyna Mietlińska ², Joanna Leszczyńska ^{2,*}, Radosław Bonikowski ² and Kamil Szymczak ^{2,*}

¹ Department of Pathophysiology, Institute of General and Experimental Pathology, Medical University of Lodz, Żeligowskiego 7/9, 90-752 Lodz, Poland; mateusz.aninowski@umed.lodz.pl

² Institute of Natural Products and Cosmetics, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Stefanowskiego 2/22, 90-537, Lodz, Poland; katarzyna.mietlinska@p.lodz.pl (K.M.); radoslaw.bonikowski@p.lodz.pl (R.B.)

* Correspondence: kamil.szymczak@p.lodz.pl; Joanna.leszczynska@p.lodz.pl

Abstract: The aim of this research was to select apple varieties suitable for allergy sufferers and diabetics. We first conducted a comprehensive analysis of several apple varieties. The total polyphenol content, sugar content, acidity, and antioxidant activity of the apple fruit juices were determined using spectrophotometric techniques. We also measured the amounts of polyphenols in the apple skins and their antioxidant potential, as well as the allergenic content in the apple juices. The strength of sensitization was assessed using the ELISA method. The lowest amounts of Bet v 1 homologues were found in the varieties Kosztela ($4.21 \pm 0.71 \mu\text{g/g}$), Koksza Pomarańczowa ($4.24 \pm 0.08 \mu\text{g/g}$), and Reneta z Brownlee ($4.86 \pm 0.18 \mu\text{g/g}$). Low values for profilin were observed for Renet Harbert ($1.74 \pm 0.22 \text{ ng/g}$) and Schieblers Taubenapfel ($2.26 \pm 0.12 \text{ ng/g}$). The highest values for profilins and Bet v 1 homologues were found in the Złotka Kwidzyńska variety ($12.40 \mu\text{g/g}$), followed by Malinowa Oberlandzka ($9.86 \mu\text{g/g}$) and Jakub Lebel ($8.87 \mu\text{g/g}$). The varieties richest in profilin were Reneta Blenheimska (8.81 ng/g), Szara Reneta (7.88 ng/g), Pepina Ribston (7.67 ng/g), and Reneta z Brownlee (7.63 ng/g). Given their minimal content of both profilins and Bet v 1 homologues, Koksza Pomarańczowa ($4.24 \pm 0.08 \mu\text{g/g}$ Bet v 1 and $4.49 \pm 0.82 \text{ ng/g}$ profilins) and Książę Albrecht Pruski ($5.57 \mu\text{g/g}$ Bet v 1 and 3.34 ng/g profilins) were identified as suitable for people with allergies. For diabetics, the most suitable apple variety was found to be Jakub Lebel, providing large doses of antioxidants and polyphenols (41.1 ± 2.0 and 5.16 ± 0.42 , respectively) and a relatively low sugar content (9.06). Similar results were measured for Kingfish, with 10.2 sugars, 5.68 ± 0.49 antioxidants, and 46.2 ± 1.5 polyphenols. The Galowany Pipping variety was found to have a low sugar content (9.71), corresponding to a perceived sweetness of 2.5. Prince Albert of Prussia apples had high antioxidant potential (5.70 ± 0.77) and a low sugar content (11.09). The James Grieve apple variety provided a high polyphenol content of 63.3 ± 1.1 with a high antioxidant level of 5.34 ± 0.45 . Grochówka offers a combination of a high polyphenol content (48.6 ± 1.9), a high level of antioxidant protection (5.50 ± 0.34), and a very low perceived sweetness (from 3.0).

Keywords: apples; polyphenols; allergenic proteins; allergy; diabetes

1. Introduction

Diabetes is reported to affect over 450 million people worldwide, and this figure is expected to increase to 700 million by 2045. Diabetes is a global health problem, characterized by a set of metabolic disorders including insulin resistance, hyperglycemia, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), central obesity, cardiovascular diseases, and hypertension [7]. It has long been known that unhealthy diets contribute to the incidence of non-communicable so-called “diseases of civilization”, including diabetes. These diseases are often major contributors to mortality and morbidity [8]. In response, contemporary strategies at both the population and individual levels are focused on risk prevention and the development of personalized dietary solutions. This approach is based on the understanding that modifiable lifestyle factors, particularly diet and nutrient intake, play a significant role in the etiology and progression of these diseases [9]. It also assumes that universal

dietary recommendations will work similarly in all individuals, and therefore in the population. However, this approach often ignores differences between individuals in terms of dietary needs. Taking these differences into account opens the possibility of achieving better health outcomes in the general population [10–12].

The apple (*Malus domestica* L. Borkh), a fruit of the rose family (*Rosaceae*), is cultivated and consumed all over the world [1]. Apple tree fruits are a rich source of nutrients and nutraceuticals and recommended as a part of a healthy diet. Eating apples can also reduce the risk of cardiovascular disease and type II diabetes [14]. Apples are one of the fruits with the highest polyphenol content [3]. Both the peel and the pulp are rich in polyphenols, including catechins, quercetin, rutin, phlorizin, phloretin, and chlorogenic acid, which have positive effects on health, especially limiting the development of neurodegenerative diseases. In vitro studies on mice have shown that polyphenols extracted from apples can have a protective effect on gastrointestinal mucosa damaged by drugs [3]. Many other in vitro studies and clinical trials have demonstrated that polyphenol-rich foods such as apples can have positive biological effects on human and animal health, especially by stimulating the immune system [4]. In particular, the outer layer of the apple contains enzymatic and non-enzymatic antioxidants, which are valuable for maintaining homeostasis [4]. Polyphenols are known for their potential to reduce the allergenicity of various food sources, including apples [13]. Not only does their anti-inflammatory effect have a beneficial effect on allergic reactions, but they can also interact directly with food allergens and weaken their allergenicity.

However, despite their high polyphenol content, apples can cause allergies [2]. Allergies to fruits like apples are predominantly associated with pollinosis. In North and Central Europe, sensitization to apples is caused mainly by cross-reactive birch pollen aeroallergen, whereas in the Mediterranean area of Europe apple allergy is mostly associated with allergies to peach. The allergenicity of apples differs across cultivars [2]. Allergic reactions to apple fruit are also exhibited by people allergic to birch pollen, due to cross-reaction between the main birch pollen allergen Bet v1 and its structural homologue Mal d 1, which is the main apple allergen. Both belong to the pathogenesis-related protein 10 (PR-10) family, indicating that they play an important role in plant defense. Mal d 1, a heat-labile protein sensitive to pepsin digestion, causes relatively mild and local symptoms including itching, tingling, or swelling of the lips, tongue, and throat, known as oral allergy syndrome (OAS). In southern Europe, apple allergy is not associated with birch pollen allergy, and the symptoms are more severe. This type of allergy may be related to the non-specific lipid transfer protein (nsLTP) Mal d 3, which is resistant to high temperature and to enzymatic digestion. Other allergens in apples include profilin Mal d 4 and thaumatin-like protein Mal d 2 [5,6].

In this study, we examined different varieties of apples in terms of their health-promoting properties, sensory characteristics, and allergen content. Identifying apple varieties with reduced allergenicity and low sugar content could help diabetics and allergy sufferers. The results could help inform dietary recommendations for patients with allergies or diabetics, as it is possible to distinguish food products with lower or higher sugar content and allergenicity.

2. Materials and Methods

2.1. Fruit material and sampling design

The apple varieties analyzed in this study were sourced from the Institute of Horticulture in Skierniewice, home to one of Poland's most extensive collections of cultivated apple varieties. These selections were gathered during 2018–2019, and all varieties originated from the same cultivation site [15].

After being transported to the chemical laboratory, the fresh fruits were gently washed and the green stems were cut off. Each fruit was then cut into two parts, to remove the stalk, seeds, seed shells, seed chamber, calyx, and the calyx recess. Then, 3 g of the tissue was placed in a bowl and the fruit was crushed with a plunger to extract the juice. Samples were also prepared for further analyses. Sensory evaluations were conducted on cubed samples of the fresh fruit. For analysis of acidity, the fruits were dried (peel and pulp separately).

2.2. Analysis of potential allergenicity

The following reagents were used: 3% solution of skimmed milk powder (Piątnica, Warsaw); PBS (ang. *phosphate buffered saline*); Tween 20 (Sigma-Aldrich, USA); 3 M NaOH solution (Sigma-Aldrich, USA); mouse antibodies against Bet v I (Dendritics, France), anti-mouse antibodies (conjugate with the phosphatase enzyme, produced in goat by Sigma-Aldrich, France), PNPP solution (p-nitrophenol phosphate ready solution, produced by Sigma Life Science, Poland). The main pollen allergen was Profilin-1 Bet v II (H CUSABIO, France).

The potentially allergenic protein homologous to Mal d 1 was determined using a cross-reaction with an antibody against Bet v 1. Analyses were performed according to previously described procedures [1]. Each well in 96-well polystyrene microplates (Medium F96 by Nunc, DK) was filled with 100 μ l of standard solution (standard curve), as well as samples from extracts diluted three times. The plate was placed in a refrigerator (temperature 4°C) for 12 hours. After 12 hours, 4 \times 350 μ l of Tween's PBS wash buffer was added to each of the used wells. Then, the plate was incubated for two hours after adding 400 μ l of 3% skimmed milk solution to the wells, forming a convex meniscus. The wells were washed using 4 \times 350 μ l PBS buffer with Tween. Then, 100 μ l of mouse antibodies against Bet v 1 (diluted 1000 times) were added and incubated for 1 h at room temperature. After 1 h, the plate was washed with 4 \times 350 μ l wash buffer. Goat antibodies against mouse immunoglobulins (100 μ l) conjugated with alkaline phosphatase (diluted 5000 times) were added to each well and incubated for 1 h. After this time, 4 \times 350 μ l of washing buffer was washed and 100 μ l of substrate for the pNPP enzyme was added. After 30 minutes, a blue product was formed. The reaction was stopped by adding 100 μ l of 3 M NaOH stop solution. The absorbance was read at a wavelength of 405 nm. The allergen content was calculated according to the appropriate standard curve equation.

Profilin analogues were also identified. The test was performed analogously to the indirect ELISA test for the determination of Bet v 1 analogues, but using antibodies that detect profilins.

2.3. Sensory assessment

Before sensory evaluation of the apple samples, the test subjects were familiarized with the general taste assessment scale for sweetness and sourness. They received four solutions for comparison:

1. Apple juice diluted twice with still mineral water (sweetness and sourness close to 0);
2. Apple juice diluted twice with still mineral water with the addition of malic acid to obtain a concentration of 10 g/l (sweetness close to 0, sourness up to 9);
3. Apple juice diluted twice with still mineral water with the addition of fructose to obtain a concentration of 60 g/l and sucrose to obtain a concentration of 60 g/l (acidity close to 0, sweetness up to 9);
4. Apple juice diluted twice with still mineral water with the addition of fructose to obtain a concentration of 60 g/l and sucrose to obtain a concentration of 60 g/l and with the addition of malic acid to obtain a concentration of 10 g/l (sweetness and sourness close to 9).

The experts were asked to lightly rinse their mouths with each solution, spit it out, then drink a glass of water. After waiting 15 minutes, they received the next apple sample for evaluation.

2.4. Determination of Acidity

The following reagents were used: 0.1 M aqueous NaOH solution cz. d. a., Chempur, Poland. The acidity of the apple was determined using the PN-EN 12147:2000 standard. The apple was washed under running water and wiped dry, then cut into small pieces (with the skin). Next, 5 g of fruit was ground in a mortar. After fine grinding, 2 g of the sample was transferred to a beaker, 25 ml of distilled water was added, and titration was performed with 0.1 M aqueous NaOH solution. An automatic titrator was used (SI Analytics TitroLine Easy). Based on a previous study [16], the end point of the titration was pH value = 8.1. The analyses were repeated twice or three times in the case of large differences between values.

2.5. Determination of sugars

The following reagents were used: methanol, 99.8% GC (Sigma-Aldrich, Poland); pyridine, 99% (Sigma-Aldrich, Poland); N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane, BSTFA + TMCS (99:1) (Sigma-Aldrich, France). The dried pulp was ground in a mortar into a fine powder. Then, 0.5 g samples were placed in extraction thimbles in Soxhlet apparatuses. The samples were extracted using 60 ml of methanol (volume of the extraction chamber approx. 50 ml). The samples were then evaporated to dryness on a rotary evaporator. The flasks were left open until the next day, to evaporate the remaining solvent. Subsequently, 2 ml of methanol was added and digested using an ultrasonic bath until homogeneous extracts were obtained. The extracts were quantitatively transferred to 2-ml glass chromatography vials. For analysis, 20 μ l of each extract was added to chromatographic vials and evaporated to dryness under a gentle stream of nitrogen. Then, 100 μ l of pyridine and 100 μ l of BSTFA + TMCS (99:1) were added. The vials were tightly capped and placed on a heating plate at 60°C for 30 min. After this time, the samples were transferred to microinserts and placed on the tray of a Gerstel MultiPurpose Sampler (MPS 2). GC-MS analyses were performed on a Pegasus 4D device by LECO, equipped with an Agilent 7890A gas chromatograph coupled with a mass spectrometer with a time-of-flight analyzer. The compounds were separated on a BPX5 column (30 m \times 0.25 mm \times 0.25 μ m) from SGE. The carrier gas was helium at a flow of 1.5 ml/min. The temperature program began at 50°C for 2 min, followed by an increase of 5°C/min to 300°C and held for 1 min (total analysis time was 53 min). The oven temperature of the second dimension column was 5°C higher than that of the first column. The dispenser temperature was 250°C. The mass spectrometer was operated in the Electron Impact mode at -70 eV and in the scanning range (m/z) from 29 to 950 amu. The temperature of the ion source was 200°C and the transfer line was 250°C.

2.6. Determination of antioxidant potential

The following reagents were used: methanol, 99.8% (GC, Sigma-Aldrich, Poland); 2,2-diphenyl-1-picrylhydrazyl radical, DPPH \cdot (Sigma-Aldrich, Poland); trolox, 97% (Sigma-Aldrich, France). The potential antioxidant was determined using the DPPH and FRAP methods. Quantitative analyses were based on a standard curve using Trolox at the following concentrations: 5mM, 2.5mM, 1 mM, 0.5 mM, 0.25 mM, 0.1 mM. In DPPH method, 2 μ L of the tested extract was transferred to a well on a 96-well plate and 250 μ L of DPPH \cdot reagent was added. Three replicates were performed for each sample. After preparing the samples, the plate was covered to protect against oxygen access and incubated in a dark place for 30 min. After this time, absorbance was measured at a wavelength of 517 nm in a Multiscan GO device (ThermoFisher Scientific). The results were converted from the standard curve to an equivalent amount of trolox (mg TE/g). In the FRAP method, 290 μ l of a solution prepared from acetate buffer with pH = 3.6, 10 mmol/dm³ TPTZ solution and 20 mmol/dm³ FeCl₃·6H₂O solution (mixed in a volume ratio of 10:1:1) was transferred to a 96-well, to which 10 μ l of the extract of the tested sample was added and mixed. After 8 min the absorbance was measured at λ = 593 nm using a Multiscan GO instrument (ThermoFisher Scientific).

2.7. Determination of total polyphenol content

The following reagents were used: Folin–Ciocalteu reagent (Chempur, Poland); gallic acid, 99.8% (Sigma-Aldrich, Poland); sodium carbonate, Na₂CO₃, part d.a. (Chempur, Poland); methanol, 99.8% GC (Sigma-Aldrich, Poland). The total polyphenol content was determined using the Folin–Ciocalteu reagent method. Polyphenolic compounds were selected based on the literature, including the most commonly used compound gallic acid [17]. For each series of determinations, a standard curve was prepared using gallic acid at concentrations of 25 mg/l, 50 mg/l, 75 mg/l, 100 mg/l, 200 mg/l, 300 mg/l and 400 mg/l in methanol. To calculate the total polyphenol content of the extracts, 2 μ l of the tested extract was added to a well on a 96-well plate, to which 4 μ l of Folin–Ciocalteu's reagent, 40 μ l of 20% Na₂CO₃, and 250 μ l of distilled water were added. Three replicates were performed for each sample. After all samples were prepared, the plate was placed in a dark place for

30 min. After this time, absorbance was measured at a wavelength of 750 nm. After scanning the plates using a Multiscan GO camera, the results were converted using the standard curve to the equivalent amount of gallic acid (mg GAE/100g).

2.8. Statistical analysis

All results were statistically tested with a one-way analysis of variance and a post-hoc Duncan test with a significance level of $p \leq 0.05$. Statistical analysis was performed using Statistica Version10 (StatSoft, Tulsa, OK). The Pearson r correlation coefficient was also used to develop the research results.

3. Results

3.1. Allergenicity results

The highest content of Bet v 1 homologues was found in the varieties Złotka Kwidzyńska (12.40 $\mu\text{g/g}$), Malinowa Oberlandzka (9.86 $\mu\text{g/g}$), and Jakub Lebel (8.87 $\mu\text{g/g}$). The varieties with the highest profilin content were Reneta Blenheimska (8.81 ng/g), Szara Reneta (7.88 ng/g), Pepina Ribstona (7.67 ng/g), Szara Reneta (7.64 ng/g), and Reneta z Brownlee (7.63 ng/g). A clear correlation was observed between the contents of individual allergens in each variety. The Pearson r correlation coefficient for the two sets was 0.55, which suggests a clear positive correlation (the higher the content of Bet v 1 homologues, the higher the profilin content). The closer to 1, the stronger the interaction between the results (Table 1). Kosztela and Kantówka Gdańska had the lowest content of Bet v 1 homologues (4.21 and 4.68 $\mu\text{g/g}$, respectively). Reneta Harberta and Schieblers Taubenapfel had the lowest content of profilins (1.74 and 2.26 ng/g, respectively) (Table 1).

Table 1. Allergen content (Bet v 1 homologs and profilins) in apple juice. All analyzed varieties were gathered in the 2019 season. The results are presented as mean \pm standard deviation.

Variety	Content of Bet v 1 homologs [$\mu\text{g/g}$]	Content of profilins [ng/g]
Boskoop	6.45 \pm 0.39	3.41 \pm 0.23
Deans' Codlin	6.48 \pm 0.28	2.90 \pm 0.33
Galowany Pipping	6.97 \pm 0.33	4.33 \pm 0.72
Grafsztynek Inflancki	6.85 \pm 0.34	4.94 \pm 0.45
Grochówka	5.45 \pm 0.39	5.40 \pm 0.30
Jakub Lebel	8.87 \pm 0.51	5.05 \pm 0.39
James Grieve	8.05 \pm 0.47	5.95 \pm 0.87
Kalwila Aderslebeńska	5.77 \pm 0.29	3.98 \pm 0.18
Kantówka Gdańska	4.68 \pm 0.59	5.71 \pm 0.26
Koksa Pomarańczowa	4.24 \pm 0.08	4.49 \pm 0.82
Kosztela	4.21 \pm 0.71	4.28 \pm 0.67
Kronselska	6.93 \pm 0.65	3.16 \pm 0.21
Krótkonózka Królewska	6.10 \pm 0.16	6.15 \pm 0.43
Książę Albert	6.97 \pm 0.20	7.26 \pm 0.15
Książę Albrecht Pruski	5.57 \pm 0.17	3.34 \pm 0.19
Malinowa Oberlandzka	9.86 \pm 0.77	7.37 \pm 0.29
Nieźrównane Peasgooda	8.09 \pm 0.68	6.49 \pm 0.42
Pepina Linneusza	7.34 \pm 0.21	7.21 \pm 0.72
Pepina Ribstona	6.97 \pm 0.30	7.67 \pm 0.27
Piękna z Rept	6.38 \pm 0.47	5.73 \pm 0.36
Reneta Blenheimska	5.73 \pm 0.10	8.81 \pm 0.35
Reneta Harberta	7.47 \pm 0.24	1.74 \pm 0.22
Reneta Kanadyjska	6.81 \pm 0.44	7.65 \pm 0.43

Reneta Kulona	7.59 ± 0.82	3.66 ± 0.44
Reneta Strauwalda	6.50 ± 0.52	6.74 ± 0.53
Reneta z Brownlee	4.86 ± 0.18	7.63 ± 0.39
Schieblers Taubenapfel	6.52 ± 0.63	2.26 ± 0.12
Szara Reneta	6.76 ± 0.29	7.88 ± 0.47
Złota Reneta	6.81 ± 0.15	7.64 ± 0.64
Złotka Kwidzyńska	12.40 ± 0.57	3.87 ± 0.32

Analyzing the above tables and ranking the varieties characterized by the lowest content of Bet v I homologs and profilins, the varieties are as follows:

Bet v I (4.21–5.77 µg/g):

Kosztela < Koksa Pomarańczowa < Kantówka Gdańska < Reneta z Brownlee < Grochówka < Książę Albrecht Pruski < Reneta Blenheimska < Kalwila Aderslebeńska

Profilins (1.74–3.98 ng/g):

Reneta Harberta < Schieblers Taubenapfel < Deans' Codlin < Kronselska < Książę Albrecht Pruski < Kalwila Aderslebeńska.

3.2. Sensory assessment

The apple varieties were subjected to sensory analysis. The averaged results are presented in Table 2.

Table 2. Results of sensory evaluation.

Variety	Sweet	Sour
Boskoop	3.3	6.3
Deans' Codlin	3.3	5.5
Galowany Pipping	2.5	4.3
Grafsztynek Inflancki	6.5	3.5
Grochówka	3.0	5.3
Jakub Lebel	7.4	3.4
James Grieve	7.8	2.0
Kalwila Aderslebeńska	7.5	3.5
Kantówka Gdańska	6.1	3.3
Koksa Pomarańczowa	5.4	5.7
Kosztela	6.3	4.5
Kronselska	6.5	3.5
Krótkonóżka Królewska	5.3	4.7
Książę Albert	6.6	4.2
Książę Albrecht Pruski	7.8	1.8
Malinowa Oberlandzka	7.4	2.0
Nieźrównane Peasgooda	3.0	3.5
Pepina Linneusza	4.3	3.7
Pepina Ribstona	4.5	4.0
Piękna z Rept	4.7	5.3
Reneta Blenheimska	7.3	4.8
Reneta Harberta	5.3	4.5
Reneta Kanadyjska	4.3	5.3
Reneta Kulona	7.3	3.0
Reneta Strauwalda	4.7	5.0
Reneta z Brownlee	2.4	6.8
Schieblers Taubenapfel	7.3	3.5

Szara Reneta	2.9	6.5
Złota Reneta	6.3	4.8
Złotka Kwidzyńska	6.5	3.5

According to the experts in the sensory evaluation tests, James Grieve and Książę Albrecht Pruski were the sweetest (7.8 each). The least sweet varieties were Reneta z Brownlee (2.4), Galowany Pipping (2.5) and Szara Reneta (2.9). The Reneta varieties were the sourest: Brownlee (6.8), Szara Reneta (6.5), and Boskoop (6.3). The Książę Albrecht Pruski (1.8) and Malinowa Oberlandzka (2.0) varieties were characterized by very low perceived acidity. It is recommended for people suffering from diabetes to eat fruits with a relatively low sugar content and low acidity. Based on the results of the sensory assessment, the apple varieties can be ranked in ascending order in terms of sweetness and acidity levels. The order for sweetness, ranging from 2.4 to 3.0, is as follows:

Reneta of Brownlee < Galated Pipping < Szara Reneta < Niezrównane Peasgooda, Grochówka

However, the order for acidity, ranging from 1.8 to 3.3, is as follows:

Książę Albrecht Pruski < Malinowa Oberlandzka, James Grieve < Reneta Kulona < Kantówka Gdańska

3.3. Acidity

Table 3 displays the mean acidity values for each variety analyzed, accompanied by the standard deviation to indicate variability within each variety.

Table 3. Average acidity of analyzed apple varieties.

Variety	Acidity [g malic acid equivalent/100g apple]
Boskoop	1.10 ± 0.04
Deans' Codlin	0.75 ± 0.01
Galowany Pipping	1.11 ± 0.02
Grafsztynek Inflancki	0.41 ± 0.03
Grochówka	0.84 ± 0.08
Jakub Lebel	0.52 ± 0.09
James Grieve	0.40 ± 0.03
Kalwila Aderslebeńska	0.95 ± 0.03
Kantówka Gdańska	0.45 ± 0.02
Koksa Pomarańczowa	0.58 ± 0.22
Kosztela	0.26 ± 0.02
Kronselska	0.78 ± 0.02
Krótkonóżka Królewska	0.80 ± 0.03
Książę Albert	0.82 ± 0.10
Książę Albrecht Pruski	0.76 ± 0.01
Malinowa Oberlandzka	0.42 ± 0.01
Niezrównane Peasgooda	0.68 ± 0.02
Pepina Linneusza	0.39 ± 0.02
Pepina Ribstona	0.72 ± 0.28
Piękna z Rept	0.92 ± 0.02
Reneta Blenheimiska	0.70 ± 0.01
Reneta Harberta	0.71 ± 0.02
Reneta Kanadyjska	0.98 ± 0.03
Reneta Kulona	1.14 ± 0.29
Reneta Strauwalda	0.79 ± 0.05
Reneta z Brownlee	1.14 ± 0.00
Schieblers Taubenapfel	0.78 ± 0.02

Szara Reneta	1.09 ± 0.22
Złota Reneta	0.51 ± 0.06
Złotka Kwidzyńska	0.53 ± 0.01

The highest values for acidity, expressed in terms of the mass of malic acid per 100 g of fruit, were observed for the varieties Reneta Kulona and Reneta z Brownlee (1.14 each), as well as for Galowany Pipping (1.11), Boskoop (1.10), and Szara Reneta (1.09). The Kosztela variety had the lowest content of acidic compounds (0.26). Renet apples, which are known for acidity [18], showed relatively high acidity. All varieties of Renet apples had higher than average acidity (from 0.70 in the case of Reneta Blenheim to 1.14 for Reneta z Brownlee and Kulona). Statistical analysis showed a clear correlation between the sensory assessment of apple sourness and measured acidity. The Pearson r correlation coefficient for these two sets was 0.60, suggesting a strong positive correlation. A similar statistical analysis was also carried out, but taking into account the influence of perceived sweetness, and then the Pearson r correlation coefficient was as high as 0.76. This shows a clear tendency for consumers to perceive much lower sourness in the case of apple fruit with high sweetness. Much of the remaining variability can be explained by the composition of organic volatile compounds (aroma). Interestingly, incorporating the sensory evaluation of apple aroma and overall flavor into the analysis did not notably alter the correlation coefficient.

3.4. Sugar content

The main challenge with assessing the sugar content in apples is that these compounds change very dynamically in terms of quantity as the fruit ripens. Moreover, fruit on trees ripens unevenly, depending mainly on the amount of sun and shade to which it is exposed, but also on the amount of rainfall and average air temperature. All this makes it virtually impossible to collect apple fruit samples at exactly the same level of ripeness for all seasons, varieties, trees, etc. Therefore, analysis of results of assessments even at the time of collection will be subject to some errors. Only an experienced pomologist can help in collecting apples that are as comparable in terms of ripeness as possible for testing [19].

There are three main sugars present in apples: fructose, glucose and sucrose. Their quantitative determination is possible using liquid chromatography (LC) as well as gas chromatography (GC) [20]. In this study, we applied gas chromatography. The results are given in g/100g of apple pulp and presented as the average for each variety (Table 4).

Table 4. Average sugar and sorbitol content for all analyzed varieties.

Variety	Fructose	Glucose	Xylose	Sucrose	Sorbitol	Total sugars
Boskoop	7.83	1.80	0.00	3.94	0.21	13.57
Deans' Codlin	6.70	2.02	0.01	4.27	0.44	13.00
Galowany Pipping	5.41	0.98	0.02	3.30	0.30	9.71
Grafsztynek Inflancki	5.96	1.32	0.04	4.05	0.67	11.37
Grochówka	5.49	1.22	0.03	3.93	0.41	10.66
Jakub Lebel	4.81	1.57	0.00	2.68	0.62	9.06
James Grieve	6.33	1.21	0.08	4.15	0.78	11.77
Kalwila Aderslebeoska	5.96	1.34	0.10	4.64	0.59	12.04
Kantówka Gdaoska	6.26	1.20	0.10	3.98	0.55	11.53
Koksa Pomaraoczowa	7.67	1.62	0.04	3.23	0.60	12.56

Kosztela	6.03	1.12	0.07	4.13	0.54	11.34
Kronselska	5.65	1.61	0.08	3.57	0.62	10.91
Krótkonózka Królewska	5.90	1.05	0.02	3.06	0.48	10.02
Książę Albert	4.86	1.23	0.12	4.76	0.39	10.97
Książę Albrecht Pruski	5.11	1.06	0.04	4.88	0.74	11.09
Malinowa Oberlandzka	6.97	1.32	0.04	4.38	0.66	12.70
Nieźrównane Peasgooda	6.28	1.16	0.00	5.37	0.42	12.81
Pepina Linneusza	5.74	1.32	0.07	3.16	0.51	10.28
Pepina Ribstona	5.08	0.77	0.00	6.91	0.42	12.76
Piękna z Rept	5.34	1.02	0.07	4.31	0.42	10.74
Reneta Blenheimska	5.02	0.84	0.00	6.68	0.58	12.54
Reneta Harberta	5.83	1.07	0.01	4.40	0.54	11.31
Reneta Kanadyjska	5.17	0.86	0.00	5.16	0.40	11.19
Reneta Kulona	8.46	2.90	0.00	6.20	0.51	17.56
Reneta Strauwalda	5.42	1.36	0.11	5.45	0.37	12.34
Reneta z Brownlee	4.79	0.98	0.00	4.63	0.41	10.40
Schieblers Taubenapfel	5.87	1.51	0.02	7.28	0.72	14.68
Szara Reneta	5.09	1.37	0.01	5.05	0.77	11.52
Złota Reneta	5.21	1.23	0.02	6.26	0.74	12.72
Złotka Kwidzyńska	5.37	1.09	0.10	4.28	0.65	10.84

As can be seen from Table 4, the varieties with the highest total sugar content were Reneta Kulona (17.56 g/100 g of apple pulp), Shieblers Taubenapfel (14.68 g/100 g), and Boskoop (13.57 g/100 g). The lowest values were observed for Jakub Lebel (9.06 g/100 g), Galowany Pipping (9.71 g/100 g), and Krótkonózka Królewska (10.02 g/100 g). In ascending order of total sugar content (ranging from 9.06–10.02), the best varieties for diabetics are:

Jakub Lebel < Galowany Pipping < Krótkonózka Królewska

Sorbitol had the most significant impact on perceived sweetness, with the highest correlation coefficient of 0.78. Almost no correlation was observed for sucrose (the correlation coefficient was very close to 0). The correlation coefficients for the remaining sugars were within the range of 0.30–0.34, which indicates a weak positive correlation. For diabetics, the best varieties of apples should be low in sweetness and sourness.

3.5. Polyphenol and antioxidant potential

Table 5 shows the average results for total polyphenol content and antioxidant potential of the analyzed apple varieties. A high antioxidant potential is indicated by an equivalent amount of trolox measured at 4 mM Trolox Equivalents (TE). As many as 13 varieties exceeded this value. The highest values were measured for the Książę Albrecht Pruski, Krótkonóżka Królewska and Grochówka varieties (5.70, 5.68, and 5.50 mM TE, respectively). Kosztela and Graftsytnek Inflancki were characterized by relatively low antioxidant potential (1.64, 1.92 mM TE, respectively). The highest total polyphenol contents were observed in the varieties James Grieve (63.3 mg GAE/100 g of fruit), Złota Renata (57.6 mg GAE/100 g of fruit), and Schieblers Taubenapfel (53.0 mg GAE/100 g of fruit). The Pepina Ribstona variety had the lowest polyphenol content, at only 28.6 mg GAE/100 g of fruit, followed by Galowany Pipping (28.9 GAE/100 g) and Pepina Linneusza (29.8 mg GAE/100 g). A correlation was observed between the antioxidant potential and total polyphenol content. The Pearson r correlation coefficient was 0.67, which indicates a clear positive correlation. Values for total polyphenol content were expressed as the equivalent amount of gallic acid [mg GAE/100 g of fresh weight (FW)]. The results are presented as the means for each sample \pm the standard deviation.

Table 5. Total polyphenol content of all analyzed varieties correlated with antioxidant potential.

Variety	Total polyphenol content [mg GAE/100 g of FW]	Antioxidant potential DPPH [mM TE/g]	Antioxidant potential FRAP [mM TE/g]
Boskoop	44.1 \pm 2.7	3.40 \pm 0.45	3.27 \pm 0.55
Deans' Codlin	38.6 \pm 1.2	3.33 \pm 0.47	3.45 \pm 0.67
Galowany Pipping	28.9 \pm 2.1	2.86 \pm 0.48	2.23 \pm 0.15
Graftsytnek Inflancki	33.7 \pm 1.4	1.92 \pm 0.32	1.05 \pm 0.27
Grochówka	48.6 \pm 1.9	4.42 \pm 0.18	5.50 \pm 0.34
Jakub Lebel	41.1 \pm 2.0	4.51 \pm 0.52	5.16 \pm 0.42
James Grieve	63.3 \pm 1.1	4.36 \pm 0.47	5.34 \pm 0.45
Kalwila Aderslebeńska	32.3 \pm 1.0	3.99 \pm 0.43	4.44 \pm 0.56
Kantówka Gdańska	42.6 \pm 2.3	3.80 \pm 1.32	4.02 \pm 0.29
Koksa Pomarańczowa	38.9 \pm 1.1	2.55 \pm 0.34	1.98 \pm 0.13
Kosztela	33.8 \pm 0.9	1.64 \pm 0.41	1.09 \pm 0.22
Kronselska	46.6 \pm 1.3	2.14 \pm 0.22	1.99 \pm 0.31
Krótkonóżka Królewska	46.2 \pm 1.5	4.33 \pm 0.29	5.68 \pm 0.49
Książę Albert	42.8 \pm 0.7	4.23 \pm 0.70	5.32 \pm 0.67
Książę Albrecht Pruski	51.3 \pm 2.7	4.54 \pm 0.44	5.70 \pm 0.77
Malinowa Oberlandzka	33.6 \pm 1.0	2.45 \pm 0.25	2.25 \pm 0.38
Nieźrównane Peasgooda	31.9 \pm 0.8	2.58 \pm 0.36	2.17 \pm 0.24
Pepina Linneusza	29.8 \pm 1.0	3.06 \pm 0.18	3.05 \pm 0.35
Pepina Ribstona	28.6 \pm 0.9	2.83 \pm 0.28	2.50 \pm 0.32
Piękna z Rept	30.2 \pm 1.7	3.22 \pm 0.16	3.27 \pm 0.20
Reneta Blenheimska	42.7 \pm 1.2	4.63 \pm 0.37	4.96 \pm 0.56
Reneta Harberta	40.0 \pm 1.5	3.24 \pm 0.25	3.31 \pm 0.54
Reneta Kanadyjska	50.0 \pm 1.8	4.01 \pm 0.14	4.52 \pm 0.68
Reneta Kulona	47.0 \pm 1.3	4.30 \pm 0.37	4.49 \pm 0.43
Reneta Strauwalda	40.1 \pm 1.6	4.42 \pm 0.45	4.94 \pm 0.75
Reneta z Brownlee	33.0 \pm 0.9	3.00 \pm 0.27	2.53 \pm 0.29

Schieblers Taubenapfel	53.0 ± 1.0	4.30 ± 0.38	4.80 ± 0.57
Szara Reneta	39.8 ± 0.9	4.24 ± 0.32	4.81 ± 0.61
Złota Reneta	57.6 ± 1.8	4.02 ± 0.18	4.44 ± 0.39
Złotka Kwidzyńska	51.8 ± 1.7	3.96 ± 0.53	4.06 ± 0.42

By comparing the sugar content, perceived sweetness, antioxidant potential, and polyphenol content, the most suitable apple varieties for diabetics can be identified—providing a high dose of antioxidants and polyphenols. The Jakub Lebel variety has a relatively low sugar content (9.06) and high levels of polyphenols and antioxidants (203.7 ± 10.6 and 4.51 ± 0.52 , respectively). A similar correlation was observed in Krótkonózka Królewska, with 10.2 sugars, 4.33 ± 0.29 antioxidants and 198.4 ± 1.6 polyphenols. The Galowany Pipping variety was found to have a low sugar content of 9.71, corresponding to a perceived sweetness of 2.5. Książę Albert Pruski showed a high antioxidant potential (4.54 ± 0.44), with a low sugar content (11.09). The James Grieve apple variety provides a high polyphenol content of 246.6 ± 12.4 with a high antioxidant level of 4.36 ± 0.47 . Grochówka offers a high polyphenol content of 217.0 ± 10.4 , a high level of antioxidant protection at 4.42 ± 0.18 , and a very low level of perceived sweetness at 3.0. The Pearson's r correlations for individual varieties were as follows: The correlation between DPPH and FRAP was 0.98, indicating a strong logical and consistent relationship between the two methods used to measure antioxidant potential. The correlation between TPC and DPPH was 0.60, highlighting a clear relationship between polyphenol content and antioxidant potential. The correlation between TPC and FRAP was 0.63, further emphasizing the connection between polyphenol content and antioxidant potential. Overall, these results confirm the consistent and significant relationship between polyphenol content and antioxidant potential in the tested samples.

4. Discussion

Malus domestica L. Borkh, commonly known as apples, are popular fruits renowned for their nutritional value and positive impact on health. The presence of polyphenolic compounds in apples reduces the risk of cardiovascular diseases and type II diabetes [13]. These polyphenols not only exhibit anti-inflammatory effects beneficial for allergic reactions but can also diminish the allergenicity of various food sources, including apples [21]. This study aimed to identify apple varieties suitable for allergy sufferers and individuals with diabetes.

The composition of soluble sugars such as fructose, sucrose, glucose, and sorbitol, significantly influences the quality and commercial value of fresh fruit [22]. In 2023, Shuhui Zhang and coworkers [23] investigated the regulation of the sugar transporter gene *MdSWEET9b*, which plays a pivotal role in sugar accumulation during apple development. Their findings revealed a strong correlation between sucrose and total sugar content, with sucrose holding the highest correlation coefficient at 0.867—indicating an exceptionally significant positive correlation.

The correlation analyses conducted in this study highlight the impact of different sugar components on perceived sweetness. Surprisingly, sorbitol emerged as the most influential factor, showing the highest correlation coefficient of 0.43. Sucrose exhibited practically no correlation, with a coefficient close to zero (0.007). The remaining sugars analyzed displayed weak positive correlations, with correlation coefficients ranging from 0.30 to 0.34. Consequently, the order of influence was as follows: sorbitol > glucose > fructose > sucrose. These insights contribute to a better understanding of the sweetness perception in apples, providing valuable information for both consumers and the fruit industry.

Similar results, reflecting a consistent level of total sugar content, were reported by Ticha and coworkers [24] in 2015, although different apple varieties were studied. Their study focused on the sugar composition of various apple varieties in apple homogenate and the correlation with sensory evaluation. Their purpose was to identify apple varieties suitable for individuals with obesity and diabetes. For the 17 studied varieties of apple, Ticha and coworkers reported a range of total sugars from 10.1 (Selena, Ontario) to 16.1 (Boskopske) grams per 100 grams of apple. In our study, the total

sugar content in the tested varieties varied from 9.06 (Jakub Label) to 14.68 (Schieblers Taubenapfel) per 100 grams of apples. The perceived sweetness in the study by Ticha and coworkers ranged from 12.2 (Selena) to 19.5 (Rajka, Boskopske). Our results ranged from 2.4 (Reneta with Brownlee) to 7.8 (Prince Albert of Prussia, James Grieve). The varieties recommended by Ticha and coworkers for diabetic patients were Selena and Ontario. The slight variations in the sweetness results may be explained by the subjective nature of sensory verification, conducted by different individuals with varied perceptions of sweetness.

The Jakub Lebel variety exhibited the lowest sugar content (9.06 g/100 g), while the least sweet varieties were Reneta z Brownlee (2.4) and Galated Pipping (2.5). The varieties with the lowest acidic compound content were Książę Albrecht Pruski (1.8), Malinowa Oberlandzka, James Grieve (2.0 each), Reneta Kulona (3.0), and Kantówka Gdańska (3.3). These findings provide valuable insights for individuals managing diabetes, enabling them to make informed choices about apple varieties with lower sugar levels.

Significant amounts of both Bet v 1 homologues and profilins were found in the studied apple varieties. Kosztela exhibited the lowest amounts of Bet v 1 homologues ($4.21 \pm 0.71 \mu\text{g/g}$), followed by Koksa Pomarańczowa ($4.24 \pm 0.08 \mu\text{g/g}$), Kantówka Gdańska ($4.68 \pm 0.59 \mu\text{g/g}$), and Reneta z Brownlee ($4.86 \pm 0.18 \mu\text{g/g}$). Reneta Harberta demonstrated the lowest level of Bet v 1 homologues ($1.74 \pm 0.22 \text{ ng/g}$), closely followed by Schieblers Taubenapfel ($2.26 \pm 0.12 \text{ ng/g}$). Given their minimal content of both profilins and Bet v 1 homologues, Koksa Pomarańczowa ($4.24 \pm 0.08 \mu\text{g/g}$ Bet v 1 and $4.49 \pm 0.82 \text{ ng/g}$ profilins) and Książę Albrecht Pruski ($5.57 \mu\text{g/g}$ Bet v 1 and 3.34 ng/g profilins) emerged as two varieties with noteworthy values for people with allergies.

In 2021, Siekierzyńska and coworkers [25] employed molecular and immunological methods to identify apple varieties with low allergen content. The analyzed samples included varieties investigated in the present study, such as Kosztela, Grochówka, Jakub Lebel, and Kantówka Gdańska. The results in the two studies exhibit similarities. For instance, Siekierzyńska and coworkers reported the Mal d 1 allergen content as $0.3 \mu\text{g/g}$ in Kantówka Gdańska, $0.6 \mu\text{g/g}$ in Kosztela, $9.2 \mu\text{g/g}$ in Grochówka, and $17.5 \mu\text{g/g}$ in Jakub Lebel. In our publication, the respective results were $4.68 \mu\text{g/g}$, $4.21 \mu\text{g/g}$, $5.45 \mu\text{g/g}$, and $8.87 \mu\text{g/g}$. Discrepancies can be attributed to various factors, including cultivation methods, soil conditions, storage methods, and duration [1].

Hallmann et al. in 2020 [17] found a strong correlation between Bet v 1 homologues and anthocyanins in raspberries. However, no similar correlations between individual allergens and polyphenols, or their total values, were identified in our work. In raspberries, the polyphenols are distributed evenly throughout the fruit, much like the allergens. In contrast, in apples the allergens are predominantly present in the pulp (juice), while polyphenols are primarily concentrated in the peel. The polyphenol content in apple pulp is relatively low. The absence of a positive correlation between allergens and polyphenols is encouraging, as it provides the opportunity to identify apple varieties with low allergen content and simultaneously high levels of health-promoting compounds [26].

Scientific research supports the notion that apple polyphenols can help alleviate allergic rhinitis, commonly known as hay fever. In a study conducted by Enomoto [27], which investigated the clinical effects of apple polyphenols on chronic allergic rhinitis through a randomized, double-blind, placebo-controlled study involving 33 individuals with chronic allergic rhinitis, a notable improvement was observed in the frequency of sneezing attacks and nasal discharge following the administration of a small or large dose of apple polyphenol extract [27]. These findings suggest that apple polyphenols can provide relief for hay fever sufferers, offering an alternative to traditional allergy medications.

The apple varieties exhibited high antioxidant potential determined by the DPPH method, and high total polyphenol content determined by the Folin-Ciocalteu method. Remarkably, 13 varieties showed a relatively high antioxidant potential value, surpassing the equivalent amount of trolox at the level of 4 mM TE. Krośniak [16] and coworkers reported significantly different results, possibly influenced by study methodologies (FRAP instead of DPPH) and the sample type (pressed apple juice rather than pulp extracts). Nonetheless, the results for many varieties were comparable. The

cultivars Piękna z Rept (4.2 mM/l), Koksa Pomarańczowa (4.2 mM/l), Kronselska (5.1 mM/l), and Kosztela (5.1 mM/l) exhibited lower antioxidant potential content. The results for these cultivars were even lower, at 3.22, 2.55, 2.14, and 1.64 mM TE, respectively. The varieties Reneta Kulona (7.1 mM/l) and Złota Reneta (7.9 mM/l) had relatively high values, which is in accordance with the results of our study (4.30 and 4.02 mM TE).

In 2011, Li Fu and coworkers [28] explored the antioxidant capacity and total phenolic contents of 62 fruits, including apples. Their results, though comparable to ours, revealed lower total polyphenol content in the tested apples (ranging from 58.12 ± 3.98 to 73.96 ± 3.52 mg GAE/100 g). We observed a broader range of polyphenol contents, from 137.4 ± 9.0 (Malinowa Oberlandzka) to 246.6 ± 12.4 mg GAE/100 g (James Grieve), almost tripling the polyphenol content in the analyzed fruits. The values for antioxidant potential reported by Li Fu and coworkers were in the range of 4.25 ± 0.27 to 5.47 ± 0.04 μ mol Trolox/g. In our study, presented in mM Trolox/100 g of fruit, the content ranged from 1.64 ± 0.41 (Kosztel) to 4.54 ± 0.44 (Książę Albrecht Pruski). The discrepancies in antioxidant potential may be attributed to differences in the study methodologies. Notably, Li Fu's research analyzed apple pressed juice, not peel extracts, potentially accounting for differences in Trolox content per 100g of raw material. Both our results and those of Li Fu's team provide valuable insights for consumers, dietitians, and food policymakers regarding the antioxidant function of apple fruit.

Based on the results of our study, the apple fruit varieties ranked from lowest to highest Bet v I homologue content (4.21–5.77 μ g/g) were: Kosztela < Koksa Pomarańczowa < Kantówka Gdańska < Reneta z Brownlee < Grochówka < Książę Albert Pruski < Reneta Blenheimska < Kalwila Aderslebeńska. For proflin (1.74–3.98 ng/g), Reneta Harberta < Schieblers Taubenapfel < Deans' Codlin < Kronselska < Książę Albrecht Pruski < Kalwila Aderslebeńska. In terms of sweetness (2.4–3.0), Reneta z Brownlee < Galowany Pipping < Szara Reneta < Niezrównane Peasgooda < Grochówka. In terms of acidity (1.8–3.3), Książę Albrecht Pruski < Malinowa Oberlandzka < James Grieve < Reneta Kulona < Kantówka Gdańska.

The varieties that exhibited the lowest total sugar content per 100 g of raw material (9.71–10.02) were Jakub Lebel, Galowany Pipping, and Krótkonóżka Królewska. These apples are the most suitable varieties for individuals with diabetes. For individuals aiming to maximize antioxidant or polyphenol intake, the James Grieve variety emerges as an excellent choice, providing a high polyphenol content of 63.3 ± 1.1 coupled with a robust antioxidant level of 5.34 ± 0.45 . For those preferring low perceived sweetness (3.0), the optimal option would be Grochówka, offering 48.6 ± 1.9 in polyphenol content along with a substantial antioxidant protection level of 5.50 ± 0.34 . The Jakub Lebel variety, with a relatively low sugar content (9.06), boasts both a high polyphenol content and high antioxidant content, at 41.1 ± 2.0 and 5.16 ± 0.42 , respectively. Another noteworthy variety is Krótkonóżka Królewska, with a sugar content of 10.2, accompanied by antioxidant levels of 5.68 ± 0.49 and polyphenols at 46.2 ± 1.5 . Galowany Pipping, with a low sugar content of 9.71 and perceived sweetness of 2.5, stands out as well. Książę Albrecht Pruski combines high antioxidant potential (5.70 ± 0.77) with a low sugar content of 11.09.

5. Conclusions

This analysis provides valuable guidance for individuals managing diabetes and allergies, helping them choose apple varieties that align with their dietary needs and health considerations. For individuals dealing with health challenges, it is crucial to closely monitor levels of potentially unfavorable ingredients and to stay well-informed about products that meet their unique dietary needs.

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