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RESEARCH ARTICLE

Comparative examination of bioactive phytochemicals in quince (*Chaenomeles*) fruits and their in vitro antioxidant activity

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ABSTRACT

Abstract: *Chaenomeles* fruits are being applied more frequently, mainly due to its high content of bioactive compounds and their positive effect on health condition. Currently, they are raw materials in pharmaceutical, cosmetic and food industries. The aim of work was to characterize cultivar Cido of *Chaenomeles japonica* and two varietal hybrids: *Chaenomeles* x *californica* Gold Kalif and *Chaenomeles* x *californica* Maksim under their content of mineral, vitamins, antiradical capacity, organic acids, phenolic acids and flavonols. The content of individual macroelements varied between tested cultivars. Cido was the cultivar with the highest mineral content. In that cultivar Fe was comprised two-fold higher than in cv. Maksim, and Cu level (1.1 mg/100g) doubled the content of that element in cv. Gold Kalif. FRAP test results ranged between 379 μM FeSO4/g DM for cv. Cido and 403 μM FeSO4/g DM for cv. Maksim. Low content of amygdalin was also confirmed in all studied fruits. The highest amygdalin content was found in Cido (39.49 μg/100g), and Gold Kalif contained the fewest amygdalin (18.42 μg/100 g). All the cultivars tested were characterized by the highest content of malic acid (9.22-11.44 mg / g DM), while the main phenolic acid in quince fruits was chlorogenic acid 6953.9 - 8185.5 μg / g DM. The content of organic and phenolic acids depends on the variety of the quince. Due to the high content of phytochemicals in quince fruit and the low content of amygdalin, it seems reasonable to develop new directions of fruit application in food technology and functional food design.

Keywords: *Cheanomeles*; Macroelements; Microelements; Vitamins; Amygdalin; Organic acids; Phenolic acids; Flavonols

INTRODUCTION

The quince (*Chaenomeles*) belongs to the Rosaceae family and the apple subfamily (Pomoideae). Currently, five species belonging to the genus *Chaenomeles* are distinguished: *Chaenomeles speciosa* Nakai, *Chaenomeles thibetica* Yu, *Chaenomeles cathayensis* Schneider, *Chaenomeles sinensis* (Thouin) Koehne and *Chaenomeles japonica* Lindl, and many species of simple hybrids and obtained through multiple cross-breeding (Miao et al. 2018). The quince was originally cultivated as an ornamental plant, but later people started cultivate for its edible fruit.

The quince fruit is spherical, with irregular apple-like shapes. Their size varies, and their weight does not exceed 50 g. When immature they are green, while the mature ones become yellow and are covered with a layer of cuticle, sometimes there with a red colour. Ripe fruits are hard with a slightly sticky skin, long-lasting and strong aroma, and a tart-sour taste. The seed chamber of the quince fruit contains 50 - 80 brown seeds (Zhang et al. 2019b). After cutting, the fruit retains its original colour for a long time. Despite the thin skin, the quince fruit tolerates transport and storage well, and stays fresh for several months in cool conditions (Antoniewska et al. 2017).

Japanese quince fruit *C. japonica* are low in monosaccharides, with glucose content three times higher than fructose. The fruit also contains sucrose and sorbitol. The content of reducing sugars and their proportions familiarize the quince fruit to apricot, plum or blackberry fruit, but not to apples with which they have close kinship. Individual

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parts of Japanese quince fruit show a significant content of dietary fibre (28 ÷ 38 g/100 g of dry fruit), with the highest content in the pulp. Japanese quince fruit has dry matter content of 13-18%, depending on the climatic conditions during cultivation, and in particular the degree of sunlight (Thomas et al. 2003). Undoubtedly, the Japanese quince fruit is characteristic for its high titratable acidity of 3.5 ÷ 4.5% in terms of malic acid. These values exceed the acidity determined in blackcurrants and are comparable with lemon. Accordingly, the fruits are classified as extremely acidic, unfit for direct consumption (Xie et al. 2016). The acidity of quince fruit results from high content of organic acids, mainly malic, quinic and succinic. Juice obtained from quince fruit is also characterized by a very low pH value (2.4 ÷ 2.9), comparable to the pH of grapefruit juice (2.8 ÷ 3.0) or lemon (2.0 ÷ 2.3). Both fruits and quince juice contain a relatively high amount of vitamin C, at the level of 55 - 92 mg / 100 g of fruit, depending on the variety. Japanese quince fruit is also rich in minerals: iron, magnesium, phosphorus, zinc, molybdenum, copper (Ros et al. 2004).

Previous works showed no negative effect of processing treatments on antioxidant and nutritional properties of Japanese quince fruits (Zhang et al. 2019b). In the study of Du et al. (2013) freeze-drying warranted the highest yield of polyphenols compared with all drying methods tested. Meanwhile, maltodextrin was found to be the best biopolymer in obtaining fruit powders.

Freeze-dried quince remains its high antioxidant properties, which has been confirmed in previous works (Antoniewska et al. 2019; Turkiewicz et al. 2019).

Fruits of the *Chaenomeles* plant due to the high content of bioactive compounds and their impact on the human body are put to increasingly broad use. Currently, they are used in pharmacy, cosmetics and the food industry (Antoniewska et al. 2017; Pawlak-Lemańska et al. 2018; Antoniewska et al. 2019). In China, quince fruit is referred to as Mugua and has been present for years in the treatment of rheumatoid arthritis, hepatitis, asthma and colds. Fruits contain polyphenols, i.e. flavanols, flavones, flavonols, anthocyanins and triterpenes, including oleanolic and ursolic acid (Antoniewska et al. 2019). These compounds can be helpful in the diet therapy of cardiovascular and cancer diseases, and show antimicrobial and anti-inflammatory effects. Proanthocyanidins and two triterpenes, also having anti-inflammatory, anti-cancer and anti-hyperlipidemic effects, are responsible for the main antioxidant activity in these fruits. *In vitro* studies have shown an inhibitory effect of flavonols obtained from Japanese quince fruit on human prostate and breast cancer cells. Flavonols have been found to show a strong antiproliferative effect on cancer cells, manifested in inhibiting their invasiveness and reducing the expression level of genes involved in apoptosis, angiogenesis and metastasis (Lewandowska et al. 2013).

Quince fruits are used in the food industry, in fruit and vegetable processing, mainly as an ingredient of juices, jams and preserves. Juice is also used as an ingredient of low-energy drinks and confectionery (Antoniewska et al. 2019). Due to the high content of pectins, quince fruit is used in the production of jellies, and dried fruit is used as a flavouring ingredient for teas. The fruit was also used as a functional additive to cookies, in which they acted as an antioxidant (Antoniewska et al. 2017; Pawlak-Lemańska et al. 2018).

The aim of the study was to characterise the main variety of *Chaenomeles japonica* Cido quince and two hybrid varieties of botanist W.B. Clark: *Chaenomeles x californica* Gold Kalif and *Chaenomeles x californica* Maksim in terms of content of minerals, vitamins, phenolic acids, flavonols, organic acids and antioxidant activity. Therefore, the key objective of the work was to assess if hybrid varieties may differ from the origin variety in their antioxidant activity and content of bioactive ingredients.

MATERIALS AND METHODS

Material

The subject of the study were the fruits of the quince (Fig. 1), which came from the "Szynsad" Orchard Farm in Dąbrówka Nowa near Grójec (51º 46 '56.093 "N 20º 42' 43.068" E). It was a plantation in the fifth year of cultivation. The plant density in the orchard was 3300 pcs / ha. The soil abundance in phosphorus, potassium and magnesium was at the following level: 72.0 mg P kg-1 of soil; 125.0 mg K.kg-1 soil; 42.0 mg Mg.kg-1 soil. In the orchard, organic manure fertilisation was applied in an amount of 18 t/ha and complementary with NPK mineral fertiliser. Keiserite was also used in an amount of 100 kg of fertiliser per 1 ha of crop (25 kg MgO / ha). The plantation is pruned every year to harvest robust fruit. Excessive amounts of twigs are removed, as well as those shoots that did not bear fruit in the previous year.

Fruits after harvesting washed with distilled water and having had the stone removed were stored in frozen conditions (temperature = -28°C) until lyophilisation. Lyophilisation was performed in a CHRIST 1-4 LSC freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) under constant conditions. The condensation temperature in the freeze dryer was maintained at −28 °C, the temperature on the freeze dryer shelf at –20 °C, and the product temperature at –4 °C. The entire process was carried out under reduced

Fig 1. Fruit of: a) *Chanomeles japonica* Cido, b) *Chaenomeles x californica* Gold Kalif, c) Owoc *Chaenomeles x californica* Maksim.

pressure for 24 h. The fruits were extracted after grinding in Grindomix GM 200 (Retsch, Haan, Germany) for 180 seconds at 1700 *g* at 21°C. The fruits for the second tests were rinsed distilled water, having had the stone removed and pressed - the pressed juice was filtered through a 45µm membrane filter.

Mineralization

Two aliquots of approximately 0.25g were made from ground freeze-dried fruit, which were quantitatively transferred to Teflon vessels. 0.5 mL purified water, 5 mL 65% HNO_3 (VWR, Poland), 0.5 mL 37% HCl (from VWR, Poland) and 0.5 ml 5% H_2O_2 (Sigma-Aldrich, Poland) were added to each vessel. The prepared samples were subjected to microwave mineralisation in a closed system according to the mineralization program taking place in 5 successive stages with different parameters (temperature 160, 190, 210.50, 50° C; pressure 40bar; time 5,10,15,10,1 min; power 90, 99, 99, 0, 0%). After cooling, the mineralisates were quantitatively transferred to centrifuge tubes and diluted with purified water to 50 mL.

Mineral content assay

For the determination of Fe, Cu, P, Zn, Mg and Na content, control and measuring apparatus was used, which includes: TOPwave microwave mineralisation system (Analytik Jena, Germany), emission spectrometer with inductively coupled plasma ICP OES model: PlasmaQuant PQ9000 (Analytik Jena, Germany) and auto-sampler. For this purpose, the calibration curve method was used (Fig. 2). Calibration curves of the tested elements at the appropriate emission lines for a given element (emission lines of the tested elements: Fe - 259.940 nm, Cu - 327.396 nm, Zn - 206.200 nm, P- 213.618 nm, Mg- 279.078 nm, Na-589.592nm). In addition, an internal pattern was added to each level of the calibration curve to control the operation and stability of the system. In this case, a 260µl Yttrium solution at 25mg / L (CPAchem, France / Bulgaria) with a certified concentration of 1001.1 ± 2.8 mg / l in 2% HNO_3 was used as reference.

In the Fe, Zn, Cu and P analysis the axial direction of measurement was set, while in the Mg and Na radial analysis. The results were developed using Spect PQ 1.2.3.0 software (Analytik Jena, Germany). The quantitative results of the analysis were automatically corrected for the average values for two blank samples, mineralized similarly as the real ones. All results are given in mg/100g of freeze-dried fruit.

Quantification of vitamins

Quantification of vitamin C content was performed using UltiMate 3000 high performance liquid chromatograph (Thermo Scientific, United Kingdom) with UV detector set at 245 nm. The separation was carried out at 30° C on a Phenomenex LUNA C8 column (250 x 4.6 mm, 5µm). A mixture of acetonitrile (Sigma Aldrich, Germany): potassium hydrogen phosphate buffer solution 12:88 (v: v) at a flow rate of 1.6 mL/min was used as the eluent. Test samples applied to the column in an amount of 20 µl. The content of vitamin C was quantified based on the peak area of the external standard. The final result is expressed in mg/100g of fruit.

The quantification of vitamin B1, B2, B3 and B6 content was made using a UltiMate 3000 high performance liquid chromatograph from Thermo Scientific with a UV detector set at 275 nm. The separation was carried out at 30° C on the Phenomenex LUNA C8 (2) column (100 x 4.6 mm, 5µm). A mixture of methanol: buffer solution pH 2.8 15:85 (v: v) was used as eluent at a flow rate of 1.0 mL/min. The test samples applied to the column in an amount of 20 µl, and the sample solvent was purified water. Each trial was analysed three times. The vitamin content was expressed in mg/100g.

The quantification of vitamin B5 content was performed using a UltiMate 3000 high performance liquid chromatograph from Thermo Scientific with a UV detector set at 203 nm. The separation was carried out at 30° C on a Phenomenex LUNA C8 column (250 x 4.6 mm 5µm). A mixture of methanol (methanol: purified water 50:50): potassium hydrogen phosphate buffer solution at pH 2.6 10:90 (v: v) at a flow rate of 1.5 mL/min served as an eluent. The vitamin B5 content was expressed in mg/100g.

The quantification of vitamin A and E content was carried out using a Schimadzu high performance liquid

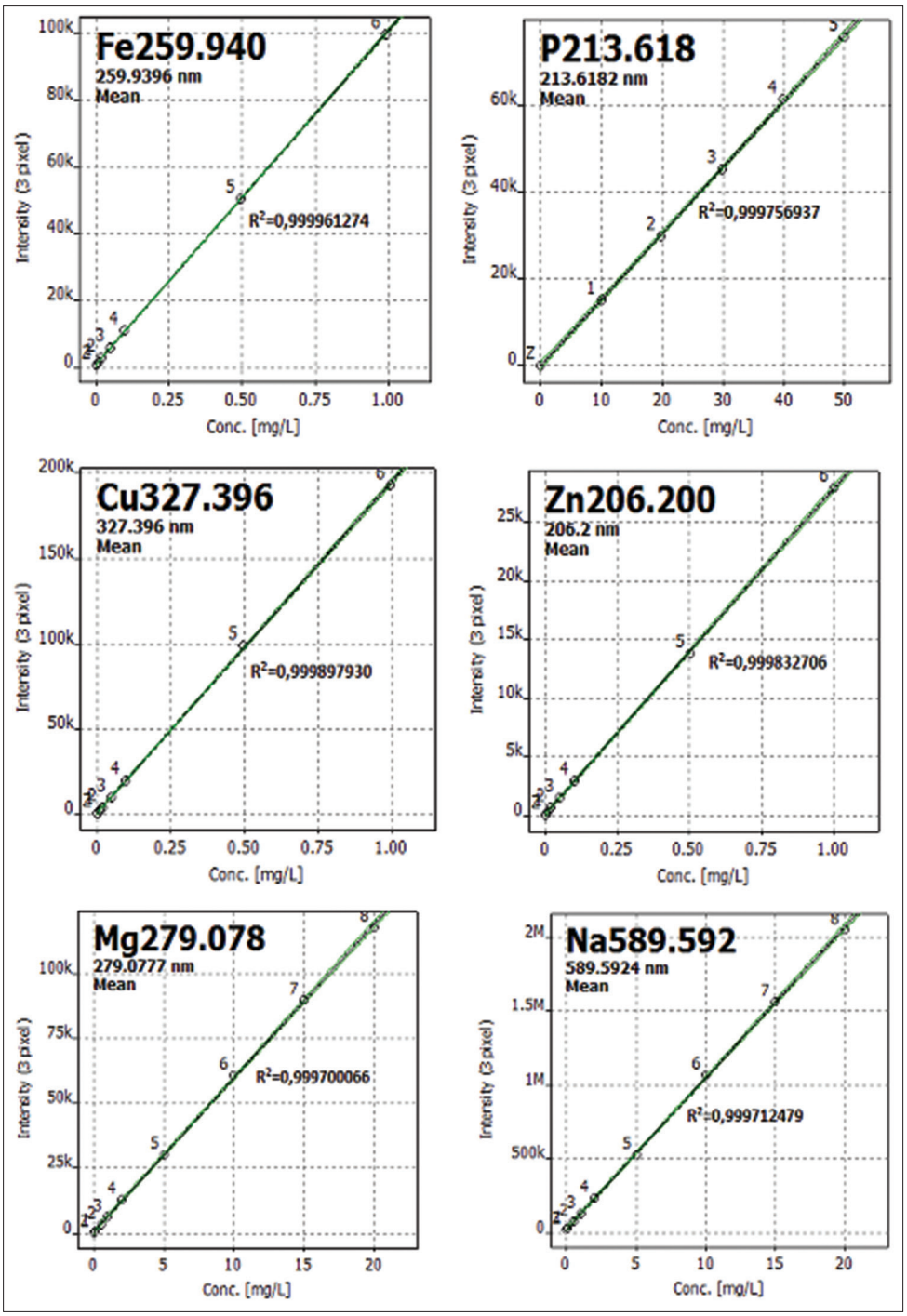

Fig 2. Calibration curves of elements assayed using ICP-OES.

chromatograph with a UV detector for vitamin E set at 285 nm and for vitamin A at 326 nm. The separation was carried out at 25° C on a Waters Spherisorb® 5.0µm ODS2 column from Waters with dimensions of 200 x 4.6 mm. A mixture of methanol and water 99: 1 (v: v) was used as eluent at a flow rate of 1.5 ml / min. Vitamin A content was expressed in µg/100g whereas vitamin E was expressed in mg/100g.

Amygdalin content assay

The amygdalin determination was carried out according to the modified methodology of Miao et al. (2013). The pressed juice was analysed, and the quantitative determination of the amygdalin content was performed with an Agilent 1260 high-performance liquid chromatograph (USA) with a UV-VIS detector. The separation was carried out at 25° C on a Phenomenex Luna column (4.6 x 250mm, 5µm). The

amygdalin content was determined using a standard curve ($r^2 = 0.989$) based on the peak areas recorded for amygdalin standard solutions (Sigam Aldrich) at λ = 214 nm. The final results are expressed in μg/100g of fruit dry matter.

Antioxidant capacity

Antiradical scavenging potential of the extract against ABTS cation radicals and DPPH radicals was analysed.

ABTS scavenging test was conducted using the modified method of Re et al. (Re et al. 1999) and described before (Kobus-Cisowska et al. 2020). DPPH method was used to evaluate the free-radical scavenging potential of the crude extract according to the method described before (Kulczyński et al. 2016). The antioxidant properties of the samples were determined using a ferric reducing/ antioxidant power assay (FRAP method) according to procedure described by O'Sullivan et al. (2013). The total polyphenols content was determined by Folin-Ciocalteu's reagent according to Cheung et al. (2003).

Determination of organic acids

The analysis of organic acids was performed using a Waters Acquity H-class UPLC system. Separation was achieved on an Acquity UPLC BEH C_{18} column (150 mm × 2.1 mm, 1.7 μm, Waters). Detection was carried out in a Waters Photodiode Array Detector (Watres Corporation, Milford, MA, USA) at λ= 280 nm as the preferred wavelength for oxalic, maleic, citric, malic, quinic and shikimic acids. The results were expressed in milligrams per gram of dry mass.

Content of phenolic acids and flavonoids.

Phenolic acids and flavonoids in tested *Chaenomeles* varieties were quantitatively evaluated according to the method described by Telichowska et al. (2020). The analysis was performed using an Acquity H class UPLC system (USA) with Waters Acquity PDA detector. As a stationary phase Acquity UPLC® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 μm) (Waters, Ireland) was used.

Statistical analysis

All assays were conducted in triplicates and results expressed as mean ± SD. One-way ANOVA testing was used to analyze statistical differences amongst the various extracts for phenolic compound contents and different antioxidant assays with least significant difference (LSD). The α value less than 0.05 was assumed as a level of significance. Correlations between the content of components and antioxidant attributes were determined by Pearson's correlation coefficients. The analysis of the principal components was used (PCA). Statistical analyses were calculated using Statistica 13.3 software (Statsoft, Poland).

RESULTS AND DISCUSSION

Content of mineral components

The analysis of metal content in the tested samples, carried out using ICP-OES, showed significant differences in the content of macro- and microelements in different varieties of quince (Table 1). Analysis of mineral components in the examined quince fruit revealed that the highest content of macro- and microelements was found in Japanese quince Cido. In contrast, hybrid varieties were characterized by lower content with varying proportions of individual micro- and macroelements.

Research has determined *Cido* to be the variety with the most minerals. In this variety there is twice as much Fe as in the Maksim variety and twice as much Cu as in the *Gold Kalif* variety. This difference may result from the size of the fruit. The quince variety *Cido* is the smallest of the studied varieties, which is why the exocarp content in the tested material is the highest, and it is the fruit peel that contains the most minerals.

Literature on the subject indicates that quince fruit is an excellent source of minerals, but also polyphenols that can be used to enrich foods (Nawirska-Olszańska et al. 2012; Antoniewska et al. 2019). Particular attention is paid to the level of magnesium, copper, zinc and phosphorus, as well as iron and molybdenum. However, the content of minerals in individual varieties can differ significantly (Zhang et al. 2019a).

The content of iron and copper is extremely important in the process of fat stabilization, because these microelements can catalyse oxidation processes (Bijami et al. 2020). Their availability creates the most dangerous reactive oxygen species, which is the hydroxyl radical. Zinc, on the other hand, shows antioxidant activity, which has been confirmed in other studies on tea leaves (Samadi and Raouf Fard 2020). Zinc has a similar antioxidant effect, which, as determined in the work, in quince fruit was present in various amounts depending on the variety (Sarpras et al. 2019).

Table 1: The content of minerals in the examined quince fruits

Mineral content (mg/100g)	variety		
	Maksim	Gold Kalif	Cido
Fe	1.44^{a}±0.11	2.18^{b}±0.10	3.29^{b}±0.10
Cu	0.73^{a}±0.04	0.54^{b}±0.03	1.10^{c}±0.01
Zn	1.12^{a}±0.11	1.41^{b}±0.15	1.44^{b}±0.10
P	357.41^{a}±20.02	153.83^{b}±14.43	332.58^{a}±8.47
Mg	93.13^{a}±12.28	92.03^{a}±1.66	110.35^{b}±1.34
Na	2.91^{a}±0.03	2.01^{b}±0.04	4.41^{c}±0.08

The mean values in the line marked with different small letters indicate the significance of differences ($p \leq 0.05$)

Content of vitamins and amygdalin

The content of vitamins and amygdalin in the examined quince plants varied (Table 2). Most vitamins were found in Cido, and the least in Maksim. The varieties Gold Kalif and Maksim had the highest level of vitamin C (111.30 and 113.16 mg/100g). For comparison, the vitamin C content in lemon juice is 30-70 mg / 100g. (Lee and Coates 1999). No significant differences in thiamine content were observed in individual varieties. The variety with the highest content of riboflavin was Gold Kalif (0.30 mg/100g). The level of niacin in the Cido and Maksim varieties was the highest at 2.04 and 2.07 mg/100g, respectively. Most lipophilic vitamins were discovered in the Cido variety (171.4 μg/100g vitamin A and 1.5 mg/100g vitamin E). Of these, the lowest vitamin A levels were found in the Maksim variety, while the vitamin E content in the Gold Kalif and Maksim varieties was comparable.

The best known antioxidant vitamins include vitamin C, β-carotene (provitamin A), vitamin A (retinol) and vitamin E (tocopherol). They demonstrate the ability to neutralize free radicals and lipid peroxides. Numerous studies have determined that most effective in the prevention of coronary artery disease is α-tocopherol, followed by ascorbic acid, retinol and β-tocopherol (Adams et al. 1999; Liu et al. 2004; Leopold 2015). Although vitamin C has weaker antioxidant properties than tannins, catechins or anthocyanins, it has a multidirectional effect and effectively "scavenges" free radicals (Shamsi et al. 2006; Korantzopoulos et al. 2007). Research by Turkiewicz et al. (2020) also confirmed that *Chaenomeles* fruit can be part of a balanced diet, providing vitamins A and E.

The quince test samples contained trace amounts of amygdalin. Its highest level was found in the Cido (39.49μg/100g), while the lowest in the Gold Kalif variety (18.42 μg/100g). Amygdalin is a compound classified as cyanogenic glycoside. This means that as a result of metabolism, it is transformed into hydrogen cyanide (Wahab et al. 2015). When administered orally, amygdalin is only 3% available in the gastrointestinal tract, but some strains of intestinal bacteria can accelerate its breakdown to hydrogen cyanide. The highest concentration of amygdalin is found in Rosaceae seeds, e.g. in cherry, apricot, peach and almond seeds (Cortés et al. 2018). The literature mentions the beneficial anti-cancer properties of amygdalin however, excess consumption of amygdalin may threaten health, especially in people with liver diseases (Makarević et al. 2016; Kolesar et al. 2018). In this case, such effect can be hardly observed due to the scarce concentration of the compound in tested juices.

Table 2: The content of selected vitamins in the examined samples of quince

Vitamin content	Variety		
	Maksim	Gold Kalif	Cido
C (mg/100g)	113.16^{b}±0.50	111.30^{b}±1.46	107.50^{a}±1,01
B_1 (mg/100g)	0.09^{a}±0.01	0.09^{a}±0.00	0.08^{a}±0.00
B_2(mg/100g)	0.26^{a}±0.01	0.30^{b}±0.00	0.26^{a}±0.01
B_3(mg/100g)	2.07^{a}±0.08	1.84^{b}±0.10	2.04^{a}±0.01
B_5(mg/100g)	0.62^{b}±0.02	0.56^{b}±0.02	0.69^{a}±0.03
B_6(mg/100g)	0.172^{a}±0.00	0.20^{b}±0.01	0.17^{a}±0.01
A(μg/100g)	112.60^{c}±0.53	130.60^{b}±1.45	171.40^{a}±0.92
E (mg/100g)	1.05^{b}±0.46	1.05^{b}±0.03	1.50^{a}±0.11
Amygdalin content (μg/100 g)	30.41^{b}±2.79	18.42^{a}±0.28	39.49^{c}±10.62

The mean values in the line marked with different small letters indicate the significance of differences ($p \leq 0.05$)

Antioxidant activity

The antioxidant potential of the quince fruit was assessed. The binding capacity of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ was measured. FRAP and TPC were determined for the tested samples, and the data is presented in Table 3.

The total polyphenol content (TPC) determined by the Folin-Ciocalteu method ranged from 17.10 mg GAE/g DM for the Maksim and 18.14 mg GAE/g DM for the Gold Kalif variety. It was also determined that all tested varieties of quince were characterized by the ability to scavenge the $DPPH^{\bullet}$ free radical. The activity of the examined quince varieties ranged from 5.22 mM TE/100g for the Gold Kalif variety to 6.34 mM TE/100g for the Cido variety. The $ABTS^{\bullet+}$ activity test showed the activity of quince varieties in the range from 3.04 for the Maksim variety to 3.54 mg TE/ 100g for the Cido variety. The reducing power of FRAP ranged from 379 μM FeSO4/g DM in the Cido variety to 403 μM FeSO4/g DM in the Maksim variety.

The quince fruit and obtained juice them contain a relatively high amount of vitamin C, which determines its antioxidant activity (Turkiewicz et al. 2019). Therefore, the right proportion and content of compounds in quince fruit can have an antioxidant effect. Tarko et al. (2010) showed a strong correlation between the content of phenolic compounds and antioxidant properties. They determined antioxidant activity TPAC measured by the ABTS assay at over 10.000 μM TE / 100g and total phenolic content at over 900 mg catechin per 100g fresh fruit. Du et al. (2013) also examined the antioxidant activity of $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP in different varieties of quince. A very strong

Table 3: Antioxidant activity of the tested quince varieties

Sample/activity	Maksim	Gold Kalif	Cido
TPC (mg GAE/g DM)	17.10^{a}±0.66	18.14^{b}±0.42	17.35^{a}±0.38
$DPPH^{\bullet}$ (mM TE/100g)	5.41^{c}±0.07	5.22^{b}±0.07	6.34^{a}±0.12
$ABTS^{\bullet+}$ (mg TE/100g)	3.04^{b}±0.04	3.12^{b}±0.08	3.54^{a}±0.09
FRAP (μM FeSO4/g DM)	403^{a}±32	392^{a}±22	379^{a}±13

The mean values in the line marked with different small letters indicate the significance of differences ($p \leq 0.05$)

correlation was observed between tests analysing the free radical scavenging capacity of $ABTS^{\bullet+}$ and FRAP. The highest scavenging capacity for $ABTS^{\bullet+}$ was in *C. speciosa* 310.55 mM TE/g and FRAP reducing power 96.84 mM TE/g.

Content of organic acids

The organic acids content in the studied quince cultivars is at a similar level (20.04 -21.62 mg /g DM) (Table 4.). In the fruit of quince the malic acid it accounted for 45-58% of the tested acids. The Cido variety has the greatest content of organic acids.

The conducted research confirms Xie's (2016) research with a high content of organic acids. The dominant organic acid in quince varieties is malic acid. Such a high content of organic acids eliminates the quince fruit for direct consumption without prior treatment, but it can act as a natural functional additive with acidifying or antioxidant properties.

Content of phenolic acids and flavonols

The content of individual phenolic acids in the tested cultivars varied. Quince fruits were characterized by the highest content of chlorogenic acid (6953.9 - 8185.5 μg / 1g DM), which for 65-70% of all phenolic acids. The content of catechin and rutin accounted for 20-25% of all tested phenolic acids. Gold kalif and Cido varieties have the highest total phenolic acids content.

Table 4: Content of organic acids

Sample/organic acid (mg/g DM)	Maksim	Gold kalif	Cido
oxalic	0.33^{a}±0.01	0.34^{a}±0.02	0.40^{b}±0.04
maleic	0.06^{a}±0.00	0.06^{a}±0.00	0.08^{b}±0.01
citric	4.37^{a}±0.02	4.35^{a}±0.03	3.59^{b}±0.04
malic	10.98^{b}±0.07	9.22^{a}±0.13	11.44^{c}±0.10
quinic	5.53^{a}±0.06	5.49^{a}±0.08	4.78^{b}±0.11
shikimic	0.66^{b}±0.03	0.59^{a}±0.03	0.75^{c}±0.05
Total	20.87	20.04	21.62

The mean values in the line marked with different small letters indicate the significance of differences ($p \leq 0.05$)

Table 5: Content phenolic acids and flavonols

Sample/phenolic acid (μg/g DM)	Maksim	Gold kalif	Cido
2,5-dihydroxybenzoic acid	89.4^{b}±2.9	57.2^{a}±4.6	20.1^{c}±4,4
4-hyrdoxybenzoic acid	15.8^{b}±3.1	6.4^{a}±1.7	19.2^{b}±2,5
syringic acid	1.5^{b}±0.1	0.7^{a}±0.2	0.3^{c}±0,2
p-coumaric acid	137.2^{b}±6.8	70.0^{a}±4.2	57.2^{c}±3.4
ferulic acid	5.9^{b}±3.6	12.8^{a}±4.5	21.7^{c}±7.6
chlorogenic acid	6953.9^{b}±29.4	7364.4^{a}±12.4	8185.5^{c}±13,8
sinapic acid	292.8^{b}±8,4	316.1^{a}±11.4	279.9^{b}±12.8
trans-cinnamic acid	158.9^{b}±23.1	336.1^{a}±9.8	187.2^{c}±7.3
vanilic acid	54.4^{b}±9.3	90.6^{a}±15.4	136.9^{c}±18.3
naringenin	32.2^{b}±4.5	20.0^{a}±1.3	59.2^{c}±4.4
rutin	926.1^{b}±9.5	846.1^{a}±13.9	1070.9^{c}±14.3
quercitin	45.6^{b}±4.4	35.0^{a}±3.6	50.3^{b}±5.3
apigenin	146.7^{b}±6.7	181.7^{a}±12.5	196.6^{a}±8.4
catechin	1722.8^{b}±18.7	2084.4^{a}±12.8	1211.2^{c}±12.2

The mean values in the line marked with different small letters indicate the significance of differences ($p \leq 0.05$)

Phenolic acids are the main bioactive compounds in *Chaenomeles*. The research carried out by Miao et al. (2018) did not show the presence of cinnamic acid in the quince, but in the study, it was present in significant amounts 158.9 - 316.1 (μg/1g DM). No gallic acid was detected in the conducted studies, while in Miao's studies, gallic acid was present in small amounts. The quantitative analysis of chlorogenic acid, catechin and epicatechin was carried out in *Chaenomeles*, the content of these acids varies depending on the *Chaenomeles* cultivars, which is also confirmed by the conducted studies (Du et al. 2013). The highest content of chlorogenic acid was found in the Cido variety (8185.5 μg / 1g DM), which accounted for 70% of all determined phenolic acids.

Flavonoids are the most numerous group of active compounds in plants. There are many reports in the literature on the content of flavonoids in plant raw materials (Kobus-Cisowska et al. 2019; Szczepaniak et al. 2019). These compounds occur in particular in the leaves and outer layers of fruit, including the skin. The structure of most flavonoids is characterized by the chromane (benzodihydropyran) system (Rajan and Muraleedharan 2017; Liu et al. 2019). In plants, flavonoids may occur separately in the form of aglycones or in a form associated e.g. with sugars as glycosides. Hydrolysis may occur during technological operations. Their transformations depend on the properties these compounds exert in *in vitro* and *in vivo* systems. Many plants with flavonoids have are recommended in the prevention and treatment of various diseases (Kobus-Cisowska et al. 2019; Granato et al. 2020).

Statistical analysis principal components (PCA)

PCA analysis was used to observe potential differences between the content of minerals, vitamins, phenyl acids, organic acids, total phenolic compounds and antioxidant activity in individual varieties of quince (Fig. 3).

The PCA (biplot) projection of the qualitative analysis results of the quince fruit for the first two components (PC1 and PC2) responsible for almost 85% variation in composition, illustrated the diversity of samples in terms of the content of active ingredients and impact of individual compounds on antioxidant activity. The location of individual tests in different parts of the graph show the diversity of the tests in terms of content of analysed compounds and antioxidant activity. The location of points close to each other on the scatter chart (Gold kalif and Maksim) proves that there are no significant differences in

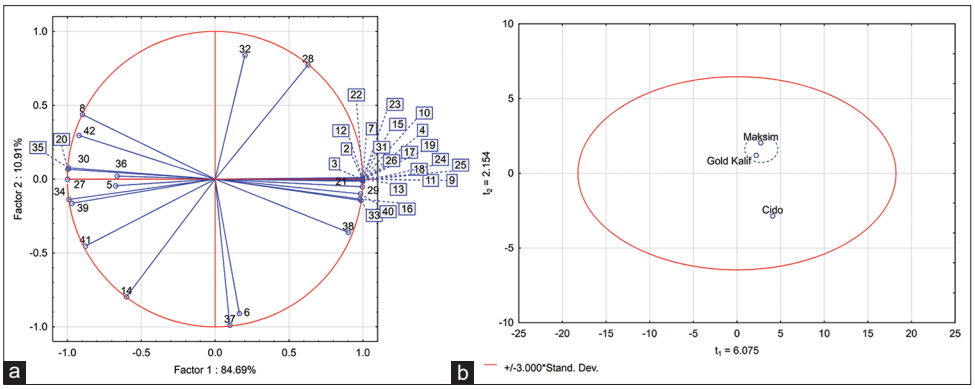

Fig 3. Principal components analysis (PCA) for quality indicators in the *Chaenomeles* fruit ingredient profile (load chart(a) and scatter plot(b)) related to the content of vitamins, minerals, phenyl acids, organic acid, TPC, amygdalin and antioxidant activity against DPPH, ABTS and FRAP. 2-Fe, 3-Cu, 4-Zn, 5-P, 6-Mg, 7-Na, 8-vitamin C, 9-vitamin B1, 10-vitamin B2, 11-vitamin B3, 12-vitamin B5, 13-vitamin B6, 14-vitamin A, 15-vitamin E, 16-amygdalin, 17- TPC Folin-Ciocalteu , 18-DPPH, 19-ABTS, 20-FRAP, 21-oxalic acid, 22-maleic acid, 23-citric acid, 24-malic acid, 25-quinic acid, 26-shikimic acid, 27-gallic acid, 28-2,5-dihydroxybenzoic acid, 29- 4-hyrdoxybenzoic acid, 30-caffeic acid, 31-syringic acid, 32-p-coumaric acid, 33-ferulic acid, 34-chlorogenic acid, 35- sinapic acid, 36- trans-cinnamic acid, 37-vanilic acid, 38-naringenin, 39-rutine, 40-quercitin, 41-apigenin, 42-catechin.

bioactive ingredients and antioxidant activity between the two hybrid varieties. However, there is a difference between hybrid and native varieties.

CONCLUSIONS

The paper analyses content minerals, vitamins, phenolic acids, flavonols and organic acids in two hybrid varieties: *Chaenomeles x californica* Gold Kalif and *Chaenomeles x californica* Maksim and the native cultivar *Chaenomeles japonica* Cido. The conducted research confirmed the differentiation in terms of the analysed components between the hybrid quince varieties and the native variety. The native variety of the Japanese quince Cido, was characterised by the highest content of bioactive compounds.

Both fruit from basic varieties and hybrid varieties can enrich the diet with antioxidants, vitamins, minerals and active ingredients. However, activity in the body must be confirmed in further studies, because the antioxidant effect results from mutual proportions of these compounds, the coexistence of other components and metabolic processes. It is also noteworthy to develop innovative opportunities to use quince fruit in the food industry as an ingredient in functional food.

Author contributions

Conceptualization, Joanna Kobus-Cisowska; Data curation, Szymon Byczkiewicz; Formal analysis, Szymon Byczkiewicz and Joanna Kobus-Cisowska; Funding acquisition, Joanna Kobus-Cisowska; Investigation, Szymon Byczkiewicz; Methodology, Szymon Byczkiewicz; Resources, Joanna Kobus-Cisowska and Piotr Szulc; Supervision, Dominik Szwajgier, Joanna Kobus-Cisowska and Piotr Szulc; Validation, Dominik Szwajgier, Joanna Kobus-Cisowska and Oskar Szczepaniak; Visualization, Szymon Byczkiewicz; Writing – original draft, Szymon Byczkiewicz and Dominik Szwajgier; Writing – review & editing, Joanna Kobus-Cisowska and Oskar Szczepaniak.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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