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DESCRIPTION AND ASSESSMENT OF CHEMICAL PROPERTIES OF FRUITS OF THE CHOCOLATE VINE (FIVE-LEAF AKEBIA) *AKEBIA QUINATA* (HOUTT.) DECNE AND DEAD MAN'S FINGERS *DECAISNEA INSIGNIS* (GRIFF.) HOKK. F. & THOMSON, GROWN IN SZCZECIN AND IN THE ARBORETUM IN GLINNA (NORTHWESTERN POLAND)

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Abstract

Originating from East Asia, the five-leaf akebia *Akebia quinata* and dead man's fingers *Decaisnea insignis* are rarely cultivated in Poland. In China, the fruits of these shrubs are a delicacy, and their skins, rich in saponins and flavonoids; are used in folk medicine. The natural range of occurrence of *Akebia quinata* and *Decaisnea insignis* is in East Asia, and the earliest mention of their cultivation in Europe dates back to 1945. Fully ripe fruits for the study were collected in September and October. The determinations included the size, color, reflactance and chemical composition (fresh berries - soluble solids, titratable acidity, pH, total polyphenol, L-ascorbic acid, nitrates and nitrites; frozen berries - phenolics and mineral composition) of fruits harvested from shrubs grown in Szczecin and in the Arboretum in Glinna near Szczecin.

In the climatic conditions of Szczecin, these species can flourish and produce ripe fruit. *Akebia quinata* bears significantly heavier fruits (72.1 g) than *Decaisnea insignis* fruits (31 g), despite similar length of fruits from both species. The fruits of *Decaisnea insignis* are more acidic (0.72 g 100 mL^{-1}) and have a higher content of polyphenols (567 mg 100 mL^{-1}), while the ones of *Akebia quinata* contain more extract (16.8%) and L-ascorbic acid (37.5 mg 100 mL^{-1}). *Deca-*

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isnea insignis have darker colour of the fruit surface (L* 18.92) and fruit flesh (L* 46.92) than fruits of *Akebia quinata* (respectively L* 25.49 and 59.93). The former species accumulated more polyphenols (*D. insignis* 567 mg, *A. quinata* 382 mg 100 mL^{-1}) in the outer parts of fruits than in the flesh (respectively 34 mg and 11 mg 100 mL^{-1}). The five-leaf *Akebia quinata* and dead man's fingers *Decaisnea insignis* are woody, ornamental plants, which can also be grown for consumption and medicinal uses.

Key words: color, fruit size, mineral composition, phenolics, reflectance.

CHARAKTERYSTYKA I OCENA WŁAŚCIWOŚCI CHEMICZNYCH OWOCÓW AKEBII PIĘCIOLISTKOWEJ *AKEBIA QUINATA* (HOUTT.) DECNE I PALECZNIKA CHIŃSKIEGO (P. FARGESA) *DECAISNEA INSIGNIS* (GRIFF.) HOKK. F. & THOMSON UPRAWIANYCH W SZCZECINIE I W ARBORETUM W GLINNEJ (PÓŁNOCNO-ZACHODNIA POLSKA)

Abstrakt

Pochodzące z Azji Wschodniej akebia pięciolistkowa i palecznik chiński są w Polsce rzadko uprawiane. W Chinach owoce tych krzewów stanowią przysmak, bogate w saponiny oraz flawonoidy skórki (osłonki) są wykorzystywane w medycynie ludowej. Naturalne stanowiska gatunków *Akebia quinata* i *Decaisnea insignis* znajdują się w Azji Wschodniej, a pierwsze wzmianki o uprawie w Europie pochodzą z 1945 roku. W pełni dojrzałe owoce do badania zebrano w trzeciej dekadzie września. W badaniach określono wielkość, barwę i refraktancję oraz skład chemiczny owoców (w świeżych – ekstrakt, kwasowość, pH, sumę polifenoli i kwasu L-askorbinowego, azotany i azotyny; w mrożonych-związki polifenolowe oraz makro- i mikroelementy), zebranych z krzewów uprawianych w Szczecinie oraz w Arboretum w Glinnej k. Szczecina. Omawiane gatunki w warunkach klimatycznych Szczecina kwitły oraz wytwarzały dojrzałe owoce. Owoce akebii chińskiej są znacznie cięższe (72,1 g) niż owoce palecznika chińskiego (31 g), pomimo zbliżonej długości. Owoce palecznika miały wyższą kwasowość (0,72 g 100 mL^{-1}) oraz zawartość polifenoli (567 mg 100 mL^{-1}), natomiast owoce akebii zawierały więcej ekstraktu (16.8%) i kwasu L-askorbinowego (37.5 mg 100 mL^{-1}). Owoce palecznika miały ciemniejszą skórkę (L*18,92) i miąższ (L*46,92) niż owoce akebii (odpowiednio L*25,49 i 59,93). Badane gatunki akumulowały kilkukrotnie więcej polifenoli (palecznik 567 mg, akebia 382 mg 100 mL^{-1}) w zewnętrznej ciemniejszej części owoców niż w miąższu (odpowiednio 34 mg i 11 mg 100 mL^{-1}). Akebia pięciolistkowa i palecznik Fargesa, drzewiaste rośliny ozdobne, mogą być również uprawiane na potrzeby konsumpcyjno-lecznicze.

Słowa kluczowe: kolor, polifenole, reflaktancja, składniki mineralne, wielkość owoców.

INTRODUCTION

The five-leaf akebia [(*Akebia quinata* (Houtt.) Decne] and dead man's fingers *Decaisnea insignis* (Griff.) Hokk. f. & Thomsonare are Asian shrub species within the family *Lardizabalaceae* Decne (*Delectis Florae* …). The natural range of occurrence of the five-leaf akeiba is in central China and Japan, where it is a common plant, and on the Korean Peninsula (KRÜSSMANN 1960, 1962, SENETA 1991), while the earliest mention of its cultivation in Europe (in England) dates back to 1945. It is a clockwise climbing creeper with

evergreen, palmate compound leaves, consisting of five entire margin leaflets. The flowers of *Akebia* are dioecious, monoecious, with a single perianth; masculine with pink perianth sepals; feminine with twice as big, reddish-purple and darker sepals (Photo 1). It usually blooms at the end of April. Several fleshy, purple when ripe, bursting, fleshy follicles (Mabberley 2008), and – according to Seneta (1991) – oblong berries, 10 cm long, create the fruit complex (syncarpium) – Photo 2. Under good conditions on plantations in China, it is possible to collect up to 30 tons of fruit in the first fruit bearing year,

Phot. 1. Male and female flowers (larger) of A*kebia quinata* (phot. M. Kubus)

Phot. 2. Aggregate fruit of *Akebia quinata* (phot. M. Kubus)

and the yield can double in subsequent years – 3 to 4 years after planting (Zhong et al. 2006). Seeds are numerous, black, immersed in white, jelly-like, sweet pulp. The seeds contain a large amount of fatty acids, mainly oleic (47.63%), palmitic (20.14%), and linoleic acid (27.05%) (Bai 2007). The fruits are edible, with chocolate-like flavor (akebia is commonly known as the 'chocolate vine'), although the skin is bitter and unpalatable. In China, culinary uses are common, e.g. unripe and grated fruits are a spice and stuffing for meat, and ripe ones are a dessert. In medicine, their diuretic, anti-inflammatory and analgesic features are valued. Dried fruits are commonly used in acupuncture (Internet 1). They contain a large amount of potassium salts and saponins and flavonoids (Madhuri, Pandey 2009). The fruits are also characterized by high antioxidant activity (Kim et al. 1994). *Akebia quinata* is listed in the Chinese Pharmacopoeia (Pharmacopoeia Commission of PRC 2005). According to Seneta (1991), in Poland, the five-leaf *Akebii quinata*

specimens are rare. During severe winters the plant freezes up to the snow line, but regenerats well. Fruits, even in areas of Poland with a mild climate, are rare.

Decaisnea insignis grows naturally in western China, in areas located in the upper reaches of the Yangtze River to the Himalayas. It is a shrub with apinnate, compound, 12-25 leaflet leaves; its leaves are entire margin ones, pointed at the distal end, bluish on the bottom. Flowers from hanging clusters of *Decaisnea insignis* are polygamous, no-leaflet ones, greenish, in the companulate form (Photo 3). The plant flowers in May and June. The fruit – fleshy, cylindrical, blue follicle – contains black seeds (Seneta 1996) – Photo 4. White, milky and viscous substance oozes from a cut fruit, whereas

Phot. 3. Flowers of *Decaisnea insignis* (phot. M. Kubus)

Phot. 4. Leaves and fruits of *Decaisnea insignis* (phot. M. Kubus)

overripe fruits disintegrate into gelatinous mass. Sweet fruits are a delicacy in China, often used to garnish desserts. In Poland, *Decaisnea insignis* is rarely grown outside botanical gardens and arboreta.

The aim of this study was to determine the quality and chemical composition of *Akebia quinata* and *Decaisnea insignis* fruits obtained from shrubs grown in northwestern Poland.

MATERIAL AND METHODS

The fruits of *Akebia quinata* came from a plant grown in a private garden on Zbójnicka Street in Szczecin, which was planted in 2001 and creeps up a wall of a three-story building with southern exposure. Since 2002, that chocolate vine plant produced a number of flowers and fruit buds (10-12 mm in diameter), which were dropped prematurely in the early years of the plant's growth. Fully ripe fruits for the study were collected in the third decade of September. It was observed that the earliest fruit buds appeared abundantly, but were shed off. It was the following, less abundant flowering period that led to the closing of the *Akebia*'s generative development cycle, producing fully developed and ripe fruits (Photo 2).

Fully ripe *Decaisnea insignis* fruits were collected in early October from several shrubs growing for over ten years in the Arboretum in Glinna near Szczecin. The plants were propagated from seeds obtained from maternal specimens growing in the Rogów Arboretum of Warsaw University of Life Sciences, incentral Poland (TUMIŁOWICZ 2005). They were planted in Glinna in 2001 and began bearing fruit in the sixth year (TUMIŁOWICZ 2013).

Physical features of fruits and soluble solids, titratable acidity, pH, total polyphenol, L-ascorbic acid, nitrates and nitrites were measured on fresh berries immediately after the harvest. The composition of phenolics and minerals was determined on samples which were kept frozen (-32°C) in polyethylene bags (2 x 500 g) until analysed.

The fruit weight was measured on a RADWAG WPX 4500 electronic scales (0.01 g accuracy). Fruit color and reflactance were measured in a transmitted mode on a Konica Minolta CM-700d spectrophotometer. Measurements were conducted in the CIE L*a*b* system through a 10° observer type and D65 illuminant, with the aperture diameter measuring 3 mm. The full nomenclature is in the 1976 CIE L*a*b* Space, International Commission on Illumination in Vienna: L* white (100) black (0), a* green (-100) red (+100), b* blue (-100) yellow (+100) (Hunterlab 2012). After harvest, the fruits were de-stemmed and crushed. Next, the colour of the pulp was determined (OCHMIAN et al. 2012*a*). To make juice, berries were macerated for 60 min. at 50°C, with the PT 400 Pektopol enzyme added in a dose of 400 mg per kg of fruits. After the enzymatic processing finished, the pulp was pressed in a hydraulic press at a pressure of 3 Mpa, after which the colour of the juice was determined (OSZMIANSKI, WOJDYŁO 2005).

The content of soluble solids was determined with a digital refractometer PAL-1 (Atago, Japan). Titratable acidity was determined by titration of a water extract with 0.1 N NaOH to the end point of pH 8.1 (measured with an multimeter Elmetron CX-732) according to PN-90/A-75101/04. L-ascorbic acid, NO_3 and NO_2 were determined with aRQflex 10 reflectometer Merck (OCHMIAN et al. 2012*a*). The total polyphenol content was estimated in methanol (70%) extracts according to SINGLETON and ROSSI (1965) with the Folin-

-Ciocalteu reagent. The data are expressed as mg of gallic acid equivalents (GAE) per 100 g of fruit tissue. In unwashed fruits, after mineralization in H_2SO_4 and H_2O_2, the total N content was determined with the Kjeldahl method. The content of K and Ca was measured with the atomic emission spectrometry, whereas the Mg content was checked with flame atomic absorption spectroscopy using an SAA Solaar device. The phosphorus content was determined with the Barton method at 470 nm wavelength, whereas the sulphur content was assayed with the turbidimetric method at 490 nm wavelength with a Marcel s 330 PRO spectrophotometer. The content of microelements (Cu, Zn, Mn, Fe), after mineralization in $HClO_4$ and HNO_3, was measured with flame atomic absorption spectroscopy using an SAA Solaar (IUNG 1972).

The values were evaluated by the Tukey's test and the differences at $p<0.05$ were considered significant. The statistical analyses were performed using the Statistica 10.0 software (Statsoft, Poland).

RESULTS AND DISCUSSION

Ripe fruits of the Chinese *Akebia quinata* were more than twice as heavy (the weight of 1 fruit – 72.1 g) as the fruits of *Decaisnea insignis* (the weight of one fruit – 31 g). Fruits of a similar length characterized both studied species, but the diameter of open follicles of *Akebia quinata* was more than twice the length of the diamater of the *Decaisnea insignis* fruits (39.2 mm vs 18.4 mm). The dimensions of *Akebia quinata* fruit collected in Szczecin are typical for this species (Li et al. 2010). The edible parts of fruits are both the skin, used mainly for pharmaceutical purposes, and the sweet pulp. The flesh of a *Decaisnea insignis* fruit made up 48.5% of the whole fruit weight. In *Akebia quinata*, the analogous percentage was 30.2%. The skin contributed as much as 62.9% to the weight of fruit, and the seeds corresponded to 6.9% of the whole fruit weight. In the wild forms of *Akebia quinata*, the skin and seeds can constitute up to 80% of the whole fruit weight (Zhong et al. 2006). In the fruits of *Decaisnea insignis,* the seeds constituted 15.6% of the total weight.The fruit of *Akebia quinata* has a delicate and sweet flavour and soft juicy texture, tasting like a mixture of banana, litchi, and passion fruit. However, the flavour varies – some fruits are fairly bland, others have more complex flavour profiles. *Akebia quinata* fruits should be harvested at optimum maturity (Li et al. 2010).

The analyses showed different content of organic and mineral components in the skins and fruit pulp of the two species (Tables 1 and 2). The percentage of extracts from *Akebia quinata* and *Decaisnea insignis* skins was similar: 9.8% and 8.3%, respectively, being significantly lower than in the fruit pulp: 13.6 and 16.8%, respectively. Pulp sugars include fructose – 4.10, glucose – 2.78, and sucrose 1.57; all given in g in 100 g (Li, Li 1991).

Table 1

Some characteristics of the analysed fruits

Testfeature	Species			
	Decaisnea insignis		*Akebia quinata*	
Physical characteristics				
Mass of 1 fruit (g)	31.0*a*		72.1*b*	
Fruit length (mm)	111*a*		104*a*	
Fruit diameter (mm)	18.4*a*		39.2*b*	
Share of skins in the fruit weight (%)	35.9*a*		62.9*b*	
Share of fruit pulp in the mass (%)	48.5*b*		30.2*a*	
Weight of seeds per fruit weight (%)	15.6 a		6.9 b	
Chemical composition				
	skin	flesh	skin	flesh
Soluble solids (%)	8.33*a*	13.61*b*	9.82*a*	16.80*c*
Juice pH	5.03*a*	5.21*b*	5.86*c*	5.82*c*
Titratable acidity (g 100 mL^{-1})	0.72*b*	0.64*b*	0.34*a*	0.28*a*
L-ascorbic acid (mg 100 mL^{-1})	29.55*a*	24.90*a*	44.20*c*	37.55*b*
Total polyphenol (mg 100 mL^{-1})	567*c*	34*a*	382*b*	11*a*
Nitrates - NO_3 (mg 1000 mL^{-1})	65.62*d*	34.23*c*	24.30*b*	11.81*a*
Nitrites - NO_2 (mg 1000 mL^{-1})	0.72*c*	0.51*b*	0.38*a*	0.33*a*

* Means marked in row with the same letter do not differ significantly at $p<0.05$ according to the Tukey's test.

The fruits were characterized by a high pH and low content of organic acids. In 100 grams of *Akebia quinata* fruits, an average of 0.31 g of acid was found, which was half the amount found in 100 g of fruit harvested from the shrubs of *Decaisnea insignis*. The acidity of fruit flesh was is generally low, which results in a high sugar-to-acid ratio (Li, Li 1991).The organic acid content in lowbush blueberry, chokeberries and blue honeysuckle ranges from 0.8 g to 1.4 g in 100 g of fruits (Ochmian et al. 2009, 2012*a*).

Fruits of neither species were rich in L-ascorbic acid. *Decaisnea insignis* had a similar content of L-ascorbic acid in its skin (29.5 mg 100 mL^{-1}) and pulp (24.9 mg 100 mL^{-1}), whereas in the *Akebia quinata* fruits, the level of L-ascorbic acid was significantly higher, especially in the skins (44.2 mg 100 mL^{-1}). Fruits of various species of *Akebia quinata*, according to Li et al. (2010), are very rich in this vitamin, containing up to 930 mg 1000 mL^{-1}. The skins of both species, especially *Decaisnea insignis* (567 mg 100 mL^{-1}), were

Table 2

The content of macro-and micronutrients in fruits of *Decaisnea insignis* and *Akebia quinata*

Mineral components		Species			
		Decaisnea insignis		*Akebia quinata*	
		skin	flesh	skin	flesh
Content (g 100 g^{-1} f.w.)	N	2.34*c*	1.60*b*	1.77*b*	1.32*a*
	P	0.42*b*	0.33*a*	0.49*c*	0.45*bc*
	K	1.58*c*	1.45*bc*	1.31*b*	1.13*a*
	Ca	0.17*c*	0.13*bc*	0.11*ab*	0.09*a*
	Mg	0.12*c*	0.09*b*	0.06*a*	0.05*a*
Content (mg 100 g^{-1} f.w.)	Cu	12.55*c*	9.46*b*	8.21*b*	5.37*a*
	Zn	11.34*c*	7.21*b*	7.89*b*	4.69*a*
	Mn	27.42*b*	30.13*b*	14.60*a*	10.14*a*
	Fe	25.26*b*	9.32*a*	31.55*b*	8.64*a*

* Means marked in rowwith the same letter do not differ significantly at $p<0.05$ according to the Tukey's test.

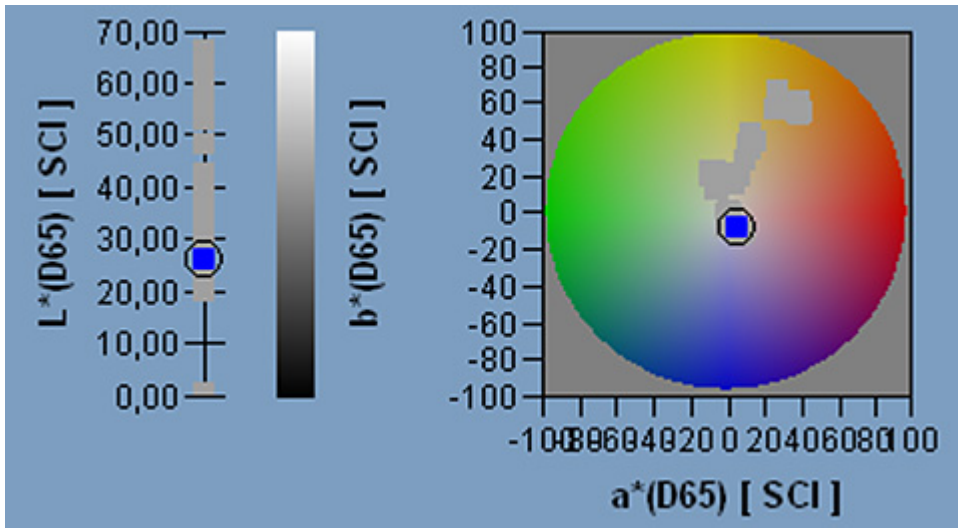
rich in polyphenolic compounds (Table 1), while the pulp was practically void of polyphenolics (*Akebia quinata* - 11, *Decaisnea insignis* 34 mg 100 mL^{-1}). This is manifested by the colour of the fruit parts as polyphenolic compounds, particularly anthocyanins, give fruit blue tinge. The skins of the analysed fruits are much darker than the pulp, as indicated by parameter L* (Table 3). Measurements also showed significant differences in the values of parameters a* and b*. They demonstrate that the skins were red-blue (Figure 1). The surface of *Akebia quinata* fruits can go from green through purple to brown in colour (Li et al. 2010). The skins of *Decaisnea insignis* fruits were darker (L* 18.92) than the skins of *Akebia quinata* fruits

Table 3

The flesh and fruit surface color of tested species

Colour CIE L*a*b*		Species	
		Decaisnea insignis	*Akebia quinata*
Color of the fruit surface	L*	18.92*b*	25.49*a*
	a*	16.02*a*	21.72*b*
	b*	-24.56*a*	-25.72*a*
Color offlesh	L*	46.92*b*	59.83*a*
	a*	0.33*a*	-3.82*b*
	b*	5.95*a*	14.41*b*

* Means marked in rowwith the same letter do not differ significantly at $p<0.05$ according to the Tukey's test.



L* (100 white, 0 black) a* (-100 green, +100 red) b* (-100 blue , +100 yellow)
Fig. 1. CIE L*a*b* chromaticity diagram

(L* 25.49), but contained less of the compounds which give a red tinge (parameter a 16.02). Parameter b* was at a similar level: 24.56 for *Decaisnea insignis* and 25.72 for *Akebiaquinata*. Also, the pulp of *Decaisnea insignis* fruits (L* 46.92) was much darker than the pulp of *Akebia quinata* fruits (L* 59.83). Parameter b* measured for the pulp of *Akebia quinata* was much higher than for the pulp of *Decaisnea insignis*. This has also been confirmed by the refractive index of individual wavelengths shown in Figure 2. Parameters L and b determined for the analysed species showed similar values to the ones reported for blue honeysuckle berries, L* 21.08; b* -25.40 (Ochmian et al. 2012*b*), and parameter L was similar in value to the one determined for fruits of the genus *Amelanchier* (Ochmian et al. 2013).

The analyses have shown that more nitrates were accumulated in the skins of both species (*Decaisnea insignis* – 47.9%, *Akebia quinata* – 52.4%) than in the pulp. Nitrites were also more abundant in the skins of *Decaisnea insignis*, while the levels of harmful nitrites in the *Akebia quinata* fruits were similar in both the skin and pulp. Generally, *Akebia quinata* fruits accumulated significantly less nitrates and nitrites than fruits of *Decaisnea insignis*. In Poland, and in many other countries, there are no regulations on a permissible nitrate content in fruits.

The analysis of the mineral content showed that in most cases the skin of the analyzed species contained more macro- and micronutrients (Table 2). The *Decaisnea insignis* fruits also contained more minerals (N, K, Ca, Mg, Cu, Zn, Mn) compared to the fruits of *Akebiaquinata*.

In the above study, the fruits of *Akebia quinata* contained much more macronutrients. In another study, by Zhang et al. (2003), the level of potas-

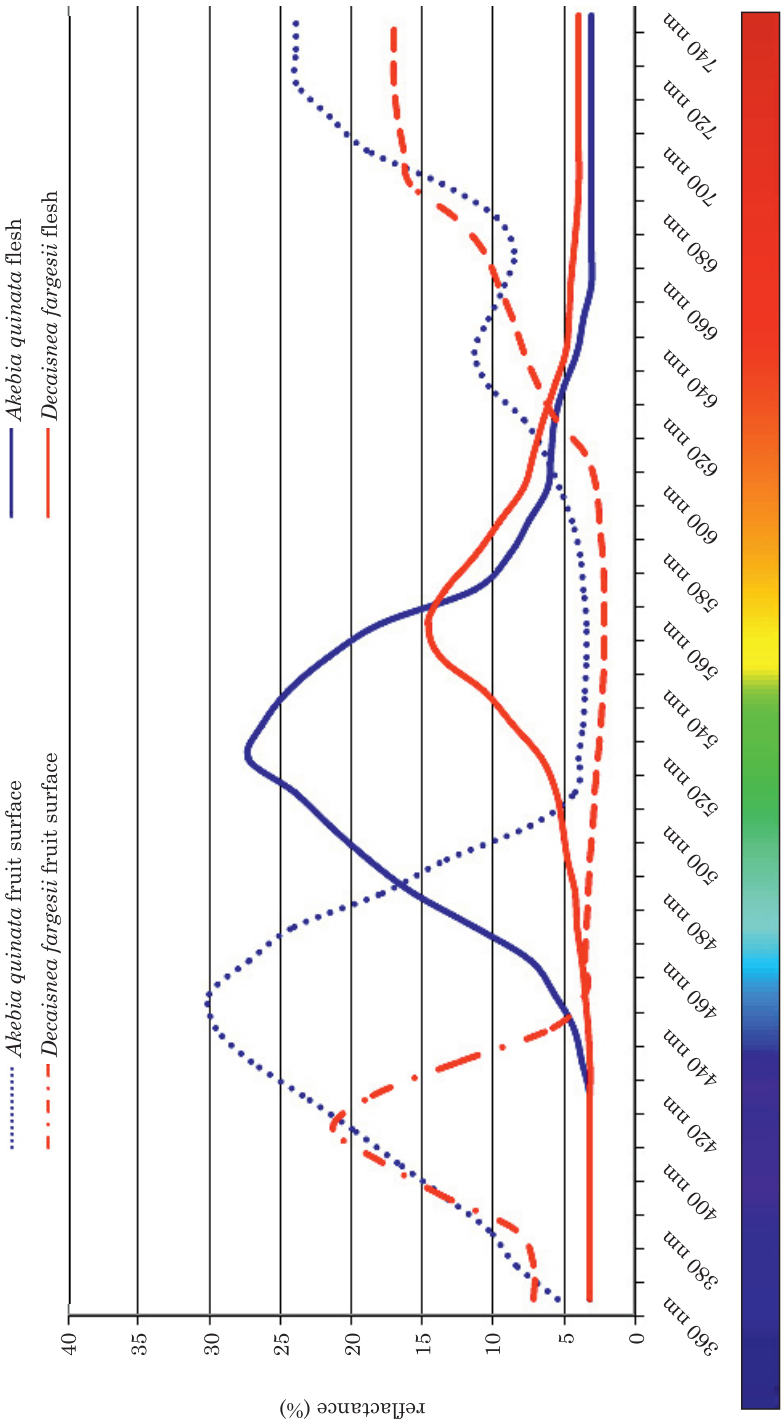


Fig. 2. Reflactance of the fruits

sium was more than double (3.21 to 4.96 g per 100 g of fresh fruit) that *Akebia quinata* fruits (1.31 and 1.13 g 100 g^{-1}). The levels of magnesium and calcium were also significantly lower. In general, concentrations of potassium, magnesium, zinc, iron, and manganese in the *Akebia quinata* species are higher than in other major dry fruits, such as apples, pears, oranges, etc. (Zhang et al. 2003). The content of macronutrients and iron in the analysed fruits was at the same level as the content of copper, while manganese was at a higher level than in fruits of several varieties of plum trees (Milošević, Milošević 2012).

CONCLUSIONS

1. The five-leaf akebia *Akebia quinata* and dead man's fingers *Decaisnea insignis* can complete the full generative phase in the climatic conditions of Szczecin, producing flowers, and then fully developed and ripe fruit.

2. The morphological traits of the analysed fruits of *Akebia quinata* and *Decaisnea insignis* correspond to the ones of the above species growing in natural conditions.

3. *Decaisnea insignis* fruits are characterized by a higher content of organic acids and polyphenols, while fruits of *Akebia quinata* contain more extract and L-ascorbic acid. In both plants, a higher content of most of these organic compounds was found in the skin or outer part of the fruit than in the pulp.

4. The skins and pulp of *Decaisnea insignis* were darker, and fruits of *Akebia quinata* contained more of the compounds, which give fruit a red and blue tinge.

5. Fruits of *Decaisnea insignis*, especially their skins, are characterized by a higher content of macro-and micronutrients, except phosphorus.

6. The five-leaf akebia *Akebia quinata* and dead man's fingers *Decaisnea insignis*, rarely grown in Western Pomerania as woody, ornamental plants, can also be cultivated for consumption and medicinal uses.

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